Nucleic Acid Detection Probes & Assay Kits

In Cells | **In Gels** | **In Solutions**

AAT Bioquest®

Our Mission

AAT Bioquest® is committed to constantly meet or exceed its customer's requirements by providing consistently high quality products and services, and by encouraging continuous improvements in its long-term and daily operations. Our core value is Innovation and Customer Satisfaction.

Our Story

AAT Bioquest®, Inc. (formerly ABD Bioquest, Inc.) develops, manufactures and markets bioanalytical research reagents and kits to life sciences research, diagnostic R&D and drug discovery. We specialize in photometric detections including absorption (color), fluorescence and luminescence technologies. The Company's superior products enable life science researchers to better understand biochemistry, immunology, cell biology and molecular biology. AAT Bioquest offers a rapidly expanding list of enabling products. Besides the standard catalog products, we also offer custom services to meet the distinct needs of each customer. Our current services include custom synthesis of biological detection probes, custom development of biochemical, cell-based and diagnostic assays and custom high throughput screening of drug discovery targets.

It is my greatest pleasure to welcome you to AAT Bioquest. We greatly appreciate the constant support of our valuable customers. While we continue to rapidly expand, our core value remains the same: Innovation and Customer Satisfaction. We are committed to being the leading provider of novel biological detection solutions. We promise to extend these values to you during the course of our service and to continue to support you with our new products and services. It is our greatest honor to receive valuable feedbacks and suggestions from you so that we can better serve your projects.

Very truly yours,

Zhenjun Diwu, Ph.D.
President

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Trademarks of AAT Bioquest

AAT Bioquest®
Apopxin™
Cell Meter™
Cell Navigator™
Cyber Green™
Cyber Orange™
CytoCalcein™
DiTO™
DiYO™
Gelite™
Helixyte™
Helixyte Green™
iFluor™
mFluor™
MitoLite™
MycoLight™
Nuclear Blue™
Nuclear Green™
Nuclear Orange™
Nuclear Red™
Nuclear Violet™
StrandBrite™
TWO-PRO™

Trademarks of Other Companies

Alexa Fluor® (Thermo Fisher)
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Pacific Orange™ (Thermo Fisher)
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RNASelect™ (Thermo Fisher)
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SYTO® (Thermo Fisher)
TO-PRO® (Thermo Fisher)
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CUSTOMER SERVICE & ORDERING INFORMATION

AAT Bioquest Corporate Headquarter:
520 Mercury Drive
Sunnyvale, CA 94085, USA
Phone: 800-990-8053 (US and Canada)
408-733-1055 (International)
Fax: 408-733-1304
Website: www.aatbio.com
E-mails: info@aatbio.com (inquire)
sales@aatbio.com (quote request)
support@aatbio.com (technical support)

International Distributors:
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TERMS AND CONDITIONS OF SALE

1. Prices, Orders and Changes: Prices shown are in US currency. Please call us for current prices if you require this information prior to placing your order. We guarantee our written quotations for 60 days. You may not cancel purchase orders unless such cancellation is expressly agreed by us. In such event, you will be advised of the total charge for such cancellation. You agree to pay such charges, including, but not limited to, storage and shipment costs, costs of producing non-standard materials, costs of purchasing non-returnable materials, cancellation costs imposed on us by our suppliers, and any other cost resulting from cancellation of this order.

2. Delivery: In most cases, we use standard overnight or two-day Federal Express delivery (or equivalent). All shipping charges billed are the responsibility of the customer and are normally prepaid by AAT Bioquest, Inc. and added to the invoice. We reserve the right to make delivery in installments, all such installments to be separately invoiced and paid for when due per invoice, without regard to subsequent deliveries. Partial shipments of available items are made when another item is backordered. Please inspect your packages upon receipt. If the goods have been damaged in transit, we can assist you in filing a claim with the carrier. You shall notify us in writing of any claims for shortages, defects or damages and shall hold the goods for our written instructions concerning disposition. Any claims for such errors must be made within 10 business days. If it is our error, we will do whatever is necessary to ship the correct products as soon as possible. If you shall fail to notify us any defects within 10 days after the goods have been received, such goods shall conclusively be deemed to conform to the terms and conditions and to have been irrevocably accepted by the buyer.

3. Payment: Terms of sale are net 30 days of date of invoice that is sent to you within 24 hours of shipping the order. The amount received must be sufficient to cover both the invoiced amount and any bank charges that may be incurred. Late charges may be added to invoices not paid within the 30-day time period. Late charges must be paid before subsequent orders can be shipped.

4. Warranties: The products shipped by AAT Bioquest are warranted to conform to the chemical or biological descriptions provided in our publications. This warranty is exclusive, and we makes no other warranty, express or implied, including any implied warranty of merchantability or fitness for any particular purpose. Our sole and exclusive liability and your exclusive remedy with respect to products proved to our satisfaction to be defective or nonconforming shall be replacement of such products without charge or refund of the purchase price, in our sole discretion, upon the return of such products in accordance with our instructions. We will not be liable for any incidental, consequential or contingent damages involving their use.

5. Returns: We must authorize any returns. We will not accept return shipments unless we have given prior written permission and shipping instructions. Goods may not be returned for credit except with our permission, and then only in strict compliance with our return shipment instructions. Any returned items may be subject to a 20% restocking fee. In many cases, items ordered in error cannot be returned because of the sensitive nature of many of our products and the difficulty and expense of requalifying returned items. If items are accepted for return, they must be in new, unopened, unused and undamaged condition, and you will be charged a per-unit 20% restocking charge.

6. Use of Our Products: Our products are used ONLY for laboratory research and development purposes. We realize that, since our products are, unless otherwise stated, intended primarily for research purposes, they may not be on the Toxic Substances Control Act (TSCA) inventory. You assume responsibility to assure that the products purchased from us are approved for use under TSCA, if applicable. You have the responsibility to verify the hazards and to conduct any further research necessary to learn the hazards involved in using products purchased from us. You also have the duty to warn your customers and any auxiliary personnel (such as freight handlers, etc.) of any risks involved in using or handling the products.

7. Patent Disclaimer: We do not warrant that the use or sale of our products will not infringe the claims of any United States or other patents covering the product itself or the use thereof in combination with other products or in the operation of any process.

8. Miscellaneous: We reserve the right to discontinue our products or change specifications or prices of our products and to correct any errors or omissions at any time without incurring obligations.

Custom Products and Services

Our Technologies

Amplite™ enzyme-based detection platform is optimized for measuring horseradish peroxidase (HRP), alkaline phosphates, luciferase, beta-galactosidase, lactamase, oxidase, protein kinases, protein phosphatases, phosphodiesterases, proteases, cytochrome P450, histone deacetylase (HDAC) and cell signaling molecules such as NAD/NADH, NADP/NADPH, IP_3, cAMP and cGMP etc.

Cell Explorer™ cell labeling platform is a complete set of tools for tracking live cells. This platform is also widely used for sorting mixed populations of cells.

Cell Navigator™ cell staining platform is a complete set of tools for selective labeling subcellular structures of live, fixed and dead cells.

Cell Meter™ cellular functional assay platform is a complete set of tools for functional analysis of cellular events and real time-monitoring of cell functions.

iFluor™ superior fluorescent labeling dyes are optimized for labeling proteins and nucleic acids. This group of dyes span from UV to infrared wavelength with good photostability and brightness.

mFluor™ superior fluorescent labeling dyes are optimized for flow cytometry applications.

PhosphoWorks™ detection platform is a set of tools for detection of ATP, ADP, AMP, phosphate, pyrophosphate, phosphoproteins and phosphopeptides.

Quest View™ colorimetric protease platform is a sensitive and robust tool for rapid detection of protease and glycosidase biomarkers. This technology platform has been licensed by a few diagnostic companies for developing rapid diagnostic tests.

RatioWorks™ superior cellular dyes are a sensitive and robust tool set for ratio imaging and real time monitoring of cellular functions (such as pH and ions) in live cells.

Screen Quest™ assay kits are a set of HTS-ready tools for high throughput screening of biochemical and cellular targets such as protein kinases, proteases, HDAC, cell apoptosis and cytotoxicity, GPCR, ion channels, ADME and transporters.

Tide Fluor™ and Tide Quencher™ superior labeling dyes are specially optimized for labeling nucleotides and peptides. This platform offers the best value in the industry. It is second to none in terms of performance and cost. This technology platform has been licensed by a few diagnostic companies for developing IVD diagnostic tests.

trFluor™ superior fluorescent labeling dyes are optimized for developing time-resolved fluorescence-based assays. It has been used for developing HTS assay technologies for many drug discovery targets.

Our Services

Besides the catalog products we also offer custom services to meet the distinct needs of each customer. Our current services include custom synthesis of biological detection probes, custom development of biochemical, cell-based and diagnostic assays, custom bioconjugation and custom high throughput screening of drug discovery targets.

Custom Assay Design and Development

At AAT Bioquest we not only make probes and assay kits, but also use them extensively ourselves. Scientists at AAT Bioquest are experts on assay design and have developed a wide variety of tests that range from biochemical detection to cellular functions. Our assay options include:

- Enzyme activities
- Binding assays
- Cell-based assays
- Microplate assays
- Flow cytometric analysis
- Fluorescence imaging

Custom Conjugation

AAT Bioquest offers the best and the most rapid bioconjugation service in the industry.

- Biotinylation
- Fluorescence labeling (iFluor™, mFluor™, APC, RPE and PerCP)
- Enzyme labeling (AP and HRP)
- Small molecule conjugation

Custom Screening

AAT Bioquest offers on-demand high-throughput screening and pharmacology profiling assays with multiple methodologies. Functional assays are designed, validated and customized to the needs of our pharmaceutical and biotechnology industry clients. These assays are aimed at assessing and monitoring the efficacy, tolerability and safety parameters of candidate compounds for treating and/or diagnosing cancer, infectious disease, autoimmunity and transplantation. Our screening options include:

- Full assay development for a target of your choice
- Optimization of your assay protocol for HTS
- Multiple assay platforms and detection methods
- Custom data analysis

Custom Synthesis of Fluorophores and Luminophores

AAT Bioquest is recognized by the top pharmaceutical companies and diagnostic companies as a key provider of novel fluorescent dyes and luminescent probes. Over the years we have developed and synthesized many enabling fluorescent and luminescent probes for running a variety of challenging biological detection tasks.

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Detecting Nucleic Acids in Cells 2

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AAT Bioquest offers a variety of fluorescent probes for detecting nucleic acids in cells. The nucleic acid stains are roughly classified into three classes that include intercalating dyes (such as ethidium bromide and propidium iodide), minor-groove binders (such as DAPI and Hoechst dyes) and nucleic acid stains of multiple action modes (such as acridine orange, 7-AAD, LDS 751 and hydroxystilbamidine, etc.). Our nucleic acid probes have been optimized to be compatible with major instrument platforms (such as fluorescence microscopes and flow cytometers).

2.1 Cell-Impermeant Nucleic Acid Detection Dyes

Thiazole Orange (TO) Analogs

The cyanine dimers, DiTO™ and DiYO™, are also referred as TOTO® and YOYO® dyes respectively (See Fig 2.1). They are symmetric dimers of cyanine dyes with exceptional sensitivity for nucleic acids. Appropriately designed dimers of nucleic acid–binding dyes have nucleic acid–binding affinities that are several orders of magnitude greater than those of their parent monomer dyes. The positively charged linker gives DiTO™ dyes a greatly enhanced affinity for nucleic acids. DiTO™ dyes exhibit a high affinity for double-stranded DNA (dsDNA) and also bind to both single-stranded DNA (ssDNA) and RNA.

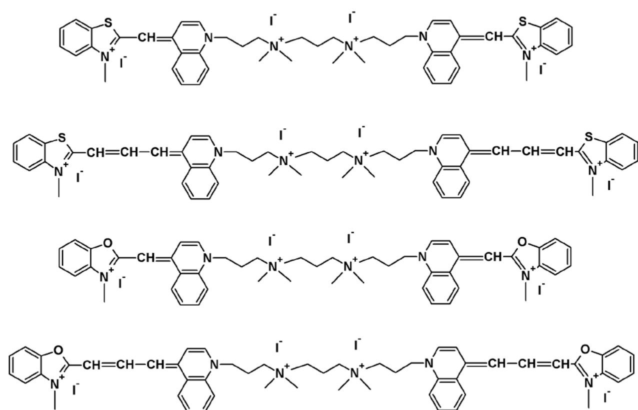

Figure 2.1. The chemical structures of DiTO™-1 (TOTO®-1, Cat# 17575), DiTO™-3 (TOTO®-3, Cat# 17576), DiYO™-1 (YOYO®-1, Cat# 17580) and DiYO-3 (YOYO®-3, Cat# 17581).

In addition to their superior binding properties, DiTO™ dyes and the other cyanine dimers (e.g., DiYO™) are essentially nonfluorescent in the absence of nucleic acids, but exhibit 100- to 1000-fold fluorescence enhancements upon DNA binding. Furthermore, the fluorescence quantum yields of the TO dimers bound to DNA are high (generally between 0.2 and 0.6), and their extinction coefficients are an order of magnitude greater than those of ethidium homodimer. This sensitivity is sufficient for detecting single molecules of labeled nucleic acids by optical imaging and flow cytometry, and for tracking dye-labeled virus particles in microbial communities and aquatic systems by fluorescence microscopy.

Simply by changing the aromatic rings and the number of carbon atoms linking the cyanine monomers, cyanine dyes can be synthesized with different spectral characteristics. DiTO™-1 dye has one carbon atom bridging the aromatic rings of the oxacyanine dye and exhibits absorption/emission maxima of 491/509 nm when bound to dsDNA. DiTO™-3 dye, which differs from DiTO™-1 dye only in the number of bridging carbon atoms, has absorption/emission maxima of 612/631 nm when bound to dsDNA. The spectra of these dyes at dye:base ratios of less than 1:1 are essentially the same for the corresponding dye–ssDNA and dye–RNA complexes. At higher dye:base ratios, however, ssDNA and RNA complexes

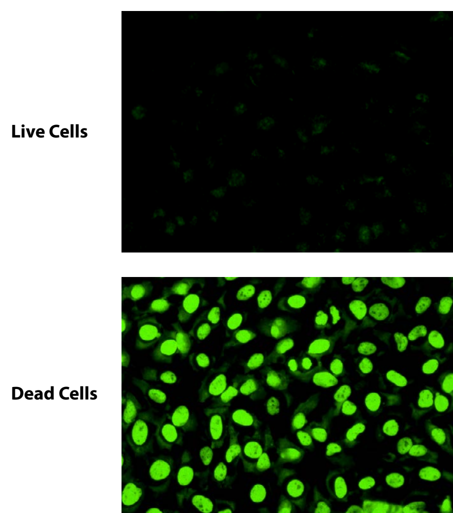

Figure 2.2. Live and dead cells stained with DiTO™-1 (Cat# 17575).

Table 2.1 Cell-Impermeant Thiazole Orange (TO) Analogs for Staining Nucleic Acids in Dead Cells

Cat #	Product Name	Size	Ex (nm)	Em (nm)
17575	DiTO™-1 [equivalent to TOTO®-1] *1 mM DMSO Solution*	0.2 mL	515	531
17576	DiTO™-3 [equivalent to TOTO®-3] *1 mM DMSO Solution*	0.2 mL	642	661
17580	DiYO™-1 [equivalent to YOYO®-1] *1 mM DMSO Solution*	0.2 mL	514	535
17581	DiYO™-3 [equivalent to YOTO®-3] *1 mM DMSO Solution*	0.2 mL	642	660
17571	TWO-PRO™ 1 [equivalent to TO-PRO® 1] *1 mM DMSO Solution*	0.2 mL	491	509
17572	TWO-PRO™ 3 [equivalent to TO-PRO® 3] *1 mM DMSO Solution*	0.2 mL	612	631

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of all the monomethine dyes of the TO series have red-shifted emissions, whereas the corresponding complexes of the trimethine analogs do not. Thus, the cyanine dimer family provides dyes with a broad range of spectral characteristics to match the output of almost any available excitation source.

DiYO™-1 dye is also capable of bis-intercalation with dsDNA and ssDNA with similarly high affinity. The binding of the dye partially unwinds the DNA, distorting and elongating the helix. At low dye:base pair ratios, the binding mode of TO and YO dyes appears to consist primarily of bis-intercalation. Each monomer unit intercalates between bases, with the benzazolium ring system sandwiched between the pyrimidines and the quinolinium ring between the purine rings, causing the helix to unwind. At high dye:base pair ratios, a second less characterized mode of external binding begins to contribute. The fluorescence emission of the DiYO™-1 dye complex with nucleic acids shifts to longer wavelengths at high dye:base ratios upon binding to single-stranded nucleic acids.

Besides TO and YO dimers, TWO-PRO™ dyes (equivalent to TO-PRO®) are also used for staining dead cells. TWO-PRO™ dyes have lower affinity for DNA than TO and YO dimers, but their DNA affinities are still significantly higher than TO, the parent compound of DiTO™, DiYO™ and TWO-PRO™ dyes. In contrast to the parent thiazole orange, DiTO™, DiYO™ and TWO-PRO™ dyes do not stain live cells (see Fig 2.3).

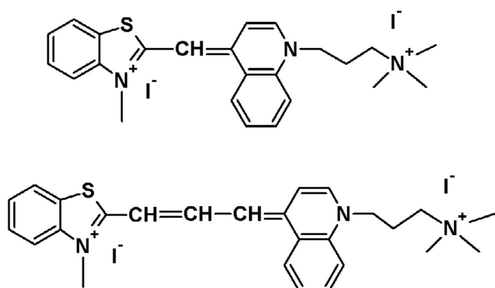

Figure 2.3. The chemical structures of TWO-PRO™ 1 (Cat# 17571) and TWO-PRO™ 3 (Cat# 17572) .

Other Cell-Impermeant Nucleic Acid Detection Dyes

7-Amino Actinomycin D (7-AAD)

7-AAD (Cat# 17501) is a non-permeant dye that can be used to identify non-viable cells. It is a fluorescent intercalator that undergoes a spectral shift upon association with DNA. 7-AAD binds selectively to GC regions of DNA, and this sequence selectivity has been exploited for chromosome banding studies. 7-AAD–DNA complexes can be excited by the argon-ion laser with emission beyond 610 nm, making this nucleic acid stain useful for multicolor fluorescence microscopy, confocal laser-scanning microscopy and immunophenotyping by flow cytometry. 7-AAD appears to be generally excluded from live cells. Cells with damaged plasma membranes or with impaired/no cell metabolism are unable to prevent 7-AAD from entering cells. Once inside cells, the dye binds to intracellular DNA producing highly fluorescent adducts which identify the cells as non-viable. Although the emission intensity of 7-AAD is lower than that of PI, the longer wavelength emission may make it widely used in flow cytometery and more useful for multiplexing assays in combination with other 488 nm-excited fluorochromes such as FITC and PE.

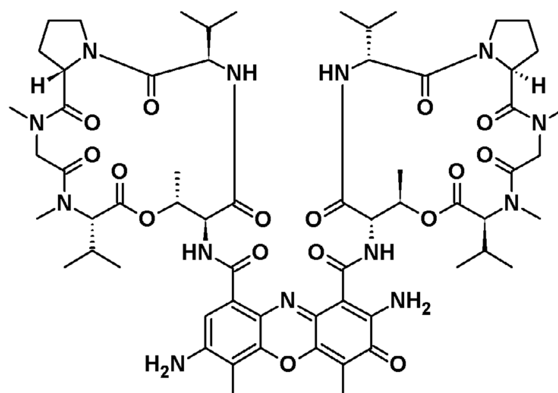

Figure 2.4. The chemical structure of 7-AAD (Cat# 17501).

Nuclear Blue™ DCS1

Our DCS1 version of Nuclear Blue™, Nuclear Orange™ and Nuclear Red™ nucleic acid stains are cell-impermeant cyanine dyes that are particularly useful as dead cell stains. They cover the full visible spectrum, and their distinct colors are convenient for multiplexing applications (see Fig 2.5 for their emission spectra). Nuclear Blue™ DCS1 (Cat# 17548) is a high-affinity nucleic acid stain that typically penetrates only cells with compromised plasma membranes. The

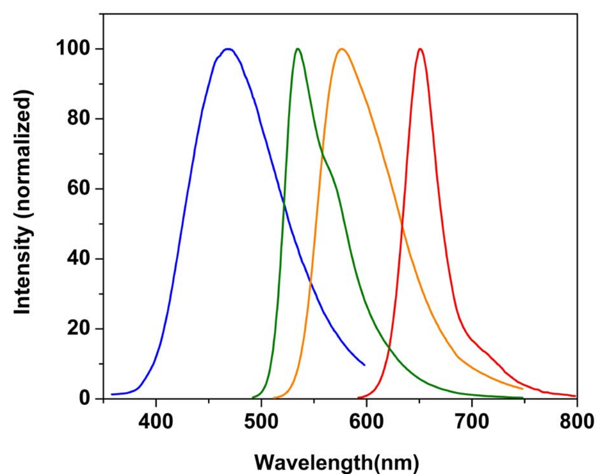

Figure 2.5. The normalized fluorescence spectra of Nuclear Blue™ DCS1(blue, Cat# 17548), Nuclear Green™ DCS1(green, Cat# 17550), Nuclear Orange™ DCS1 (orange, Cat# 17551) and Nuclear Red™ DCS1 (red, Cat# 17552) nucleic acid stains.

Nuclear Blue™ DCS1 labels dead cells with bright fluorescence centered near 460 nm. The absorption maximum of the nucleic acid–bound Nuclear Blue™ DCS1 (~350 nm) permits very efficient fluorescence excitation by the popular 350 nm UV excitation. The brightness of the Nuclear Blue™ DCS1 stain allows sensitive detection with fluorometers, microplate readers, arc-lamp–equipped flow cytometers and epifluorescence microscopes, including those not equipped with UV-pass optics.

Nuclear Green™ DCS1

Nuclear Green™ DCS1 (Cat# 17550) is a high-affinity nucleic acid stain that easily penetrates cells with compromised plasma membranes and yet will not cross the membranes of live cells. It is especially useful for staining both gram-positive and gram-negative bacteria, in which exceptionally bright signals are required. Following brief incubation with the Nuclear Green™ DCS1, dead cells fluoresce bright green when excited with the 488 nm spectral line of the argon-ion laser or with any other 450–500 nm source. Because Nuclear Green™ DCS1 is essentially nonfluorescent in aqueous medium, no wash steps are required. Unlike DAPI or Hoechst dyes, Nuclear Green™ DCS1 shows little base selectivity. These properties, combined with its ~1000-fold fluorescence enhancement upon nucleic acid binding and high quantum yield, make Nuclear Green™ DCS1 a simple and quantitative single step dead cell indicator analyzed with epifluorescence and confocal laser-scanning microscopes, fluorometers, fluorescence microplate readers and flow cytometers.

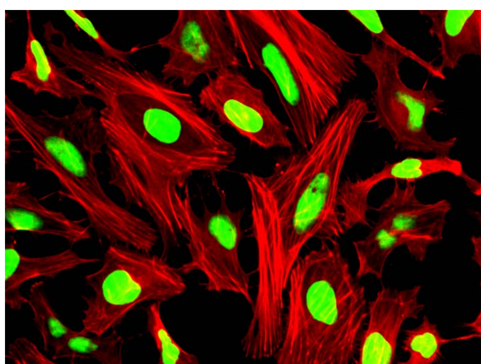

Figure 2.6. Image of HeLa cells. Actin filaments were stained with Phalloidin-iFluor™ 680 Conjugate (red, Cat# 23128), and nuclei were stained with Nuclear Green™ DCS1 (green, Cat#17550).

Nuclear Orange™ DCS1

Nuclear Orange™ DCS1 (Cat# 17551) is designed to clearly distinguish dead bacteria, yeast or mammalian cells from live cells. Compared with propidium iodide, Nuclear Orange™ DCS1 has shorter-wavelength emission and its spectra more closely match the rhodamine filter set. In addition, Nuclear Orange™ DCS1 has a much higher molar absorptivity (extinction coefficient) than propidium iodide and a far greater fluorescence enhancement upon binding DNA, suggesting that it may have a higher sensitivity as a dead-cell stain or as a nuclear counterstain. Nuclear Orange™ DCS1 might be useful for DNA fragment sizing by single-molecule flow cytometry when using a Nd:YAG laser as the excitation source.

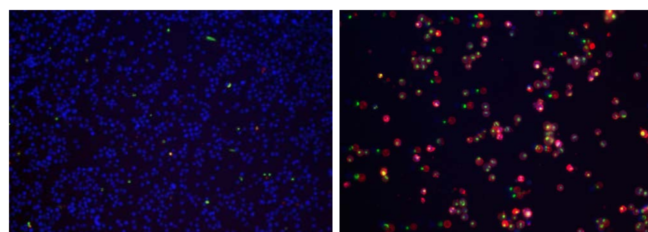

Figure 2.7. The detection of binding activity of Apopxin™ Deep Red to phosphatidylserine in Jurkat cells. The fluorescence image (right) shows live cells (blue, stained by CytoCalcein™ Violet 450, Cat# 22012), apoptotic cells (red, stained by Apopxin™ Deep Red), and necrotic cells(green, stained by Nuclear Green™ DCS1, Cat# 17550) induced by 1µM staurosporine for 3 hours. The fluorescence images of the cells were taken with Olympus fluorescence microscope using the violet, Cy5® and FITC channel respectively. Left: Non-induced control cells; Right: Triple staining of staurosporine-induced cells.

Nuclear Red™ DCS1

Nuclear Red™ DCS1 (Cat# 17552) is a fluorogenic, DNA-selective and cell-impermeant dye for analyzing DNA content in dead, fixed or apoptotic cells. Nuclear Red™ DCS1 is excited by the He-Ne laser that is extensively used in most flow cytometers. Its red fluorescence is significantly enhanced upon binding to DNA. Nuclear Red™ DCS1 can be used in fluorescence imaging, microplate and flow cytometry applications. This DNA-binding dye might be used for multicolor analysis of dead, fixed or apoptotic cells with proper filter sets.

Table 2.2 Cell-Impermeant DNA Probes for Staining Nucleic Acids in Dead Cells (Other than TO Analogs)

Cat #	Product Name	Size	Ex (nm)	Em (nm)
17501	7-AAD [7-Aminoactinomycin D]	1 mg	546	647
17548	Nuclear Blue™ DCS1	0.5 mL	350	641
17550	Nuclear Green™ DCS1	0.5 mL	503	526
17551	Nuclear Orange™ DCS1	0.5 mL	528	576
17552	Nuclear Red™ DCS1	0.5 mL	642	660
17515	Propidium Iodide *UltraPure grade*	25 mg	535	617

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Propidium Iodide (PI)

Ethidium bromide (EtBr) and propidium iodide (PI) are structurally similar to phenanthridinium intercalators. PI (Cat# 17515) is more soluble in water and less membrane-permeant than EtBr. PI is generally excluded from viable cells. It can be excited with mercury- or xenon-arc lamps or with the argon-ion laser, making it suitable for fluorescence microscopy, confocal laser-scanning microscopy, flow cytometry and fluorometry. PI dye binds with little or no sequence preference at a stoichiometry of one dye per 4–5 base pairs of DNA. PI also binds to RNA, necessitating treatment with nucleases to distinguish between RNA and DNA. Once PI is bound to nucleic acids, its fluorescence is enhanced ~10-fold, its excitation maximum is shifted ~30–40 nm to red and its emission maximum is shifted ~15 nm to blue.

Although PI molar absorptivity (extinction coefficient) is relatively low, it exhibits sufficiently large Stokes shifts to allow simultaneous detection of nuclear DNA and fluorescein-labeled antibodies, provided that the proper optical filters are used. PI is commonly used as a nuclear or chromosome counterstain and as a stain for dead cells. EtBr and PI are potent mutagens and must be handled with extreme care. Solutions containing EtBr or PI can be decontaminated by filtration through activated charcoal, which is then incinerated, thus providing an economical decontamination procedure. Alternatively, the dyes can be completely degraded in buffer by reaction with bleach.

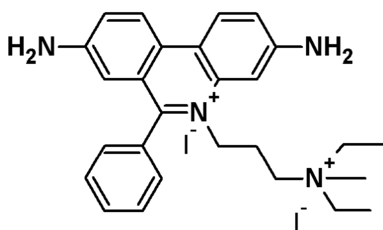

Figure 2.8. The chemical structure of propidium iodide (PI, Cat# 17515).

2.2 Cell-Permeant Nucleic Acid Detection Dyes

4',6-Diamidino-2-Phenylindole (DAPI)

DAPI is an excellent nuclear counterstain with a distinct banding pattern in chromosomes. DAPI shows blue fluorescence upon binding DNA and can be excited with a mercury-arc lamp or with UV lines of argon-ion laser. Binding of DAPI to dsDNA produces a ~20-fold fluorescence enhancement, apparently due to the displacement of water molecules from both DAPI and the minor groove. Although Hoechst dyes may be somewhat brighter in some applications, their photostability when bound to dsDNA is less than that of DAPI. Like Hoechst dyes, the blue-fluorescent DAPI apparently associates with the minor groove of dsDNA, preferentially binding to AT clusters. The DAPI–RNA complex exhibits a longer wavelength fluorescence emission maximum than the DAPI–dsDNA complex (~500 nm versus ~460 nm) but its quantum yield is only about 20%.

DAPI is quite soluble in water but has limited solubility in phosphate-buffered saline. In the presence of appropriate salt concentrations, DAPI usually does not exhibit fluorescence enhancement upon binding to ssDNA or GC base pairs. However, the fluorescence of DAPI does increase significantly upon binding to detergents, dextran sulfate, polyphosphates and other polyanions.

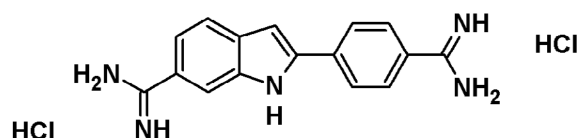

Figure 2.9. The chemical structure of 4',6-diamidino-2-phenylindole (DAPI).

Hexidium Iodide

Hexidium iodide (Cat# 17562) is a moderately lipophilic phenanthridinium dye that is permeant to mammalian cells and selectively stains almost all gram-positive bacteria in the presence of gram-negative bacteria. Hexidium iodide can be conveniently used for the discrimination of bacterial gram sign. Hexidium iodide yields slightly shorter wavelength spectra upon DNA binding than ethidium bromide or propidium iodide dyes. Generally, both the cytoplasm and nuclei of eukaryotic cells show staining with hexidium iodide, and mitochondria and nucleoli may also be stained.

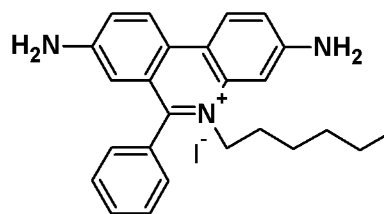

Figure 2.10. The chemical structure of hexidium iodide (Cat# 17562).

Hoechst Dyes

The bisbenzimide dyes, Hoechst 33258 (Cat# 17520), Hoechst 33342 (Cat# 17530) and Hoechst 34580 (Cat# 17537), are common nuclear counterstains. They are cell permeant, minor groove–binding DNA stains with bright blue fluorescence upon binding to DNA. Hoechst 33342 has slightly higher membrane permeability than Hoechst 33258, but both dyes are quite soluble in water (up to 2% solutions can be prepared) and relatively nontoxic. These Hoechst dyes, which can be excited with the UV spectral lines of the argon-ion laser and most conventional fluorescence excitation sources, exhibit relatively large Stokes shifts (excitation/emission maxima ~350/460 nm), making them suitable for multicolor labeling experiments. Hoechst 34580 has somewhat longer-wavelength spectra than the other Hoechst dyes when bound to nucleic acids.

Hoechst 33258 and Hoechst 33342 dyes have complex, pH-dependent spectra when not bound to nucleic acids, with much higher fluorescence quantum yields at pH 5 than at pH 8. Their

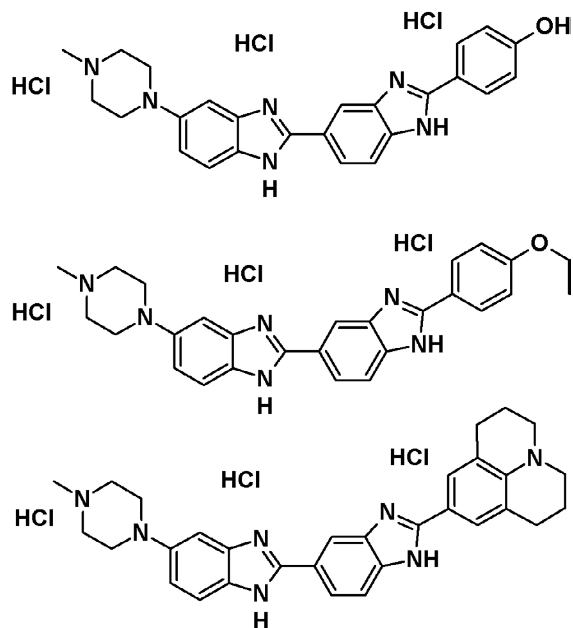

Figure 2.11. The chemical structures of Hoechst 33258 (Cat# 17520), Hoechst 33342 (Cat# 17530) and Hoechst 34580 (Cat# 17537).

fluorescence is also enhanced by surfactants, such as sodium dodecyl sulfate (SDS). These dyes appear to show a wide spectrum of sequence-dependent DNA affinities and bind with sufficient strength to poly(d(AT)) sequences, and they can displace several known DNA intercalators. They also exhibit multiple binding modes and distinct fluorescence emission spectra that are dependent on dye:base pair ratios. Hoechst dyes are used in many cellular applications, including cell-cycle and apoptosis studies. Hoechst 33258, which is selectively toxic to malaria parasites, is also useful for flow cytometric screening of blood samples for malaria parasites and for assessing their susceptibility to drugs.

Hydroxystilbamidine

The trypanocidal drug hydroxystilbamidine (Cat# 17514) is an interesting probe of nucleic acid conformation. Hydroxystilbamidine, a nonintercalating dye, exhibits AT-selective binding reported to favor regions of nucleic acids that have secondary structure. Hydroxystilbamidine has some unique spectral properties upon binding nucleic acids. At pH 5, the free dye exhibits UV excitation maxima at ~330 nm and ~390 nm, with dual emission at ~450 nm and ~600 nm. Although the red-fluorescent component remains present when bound to DNA, it is never observed when the dye is bound to RNA, permitting potential discrimination to be made between these two types of nucleic acids. The enhancement of its metachromatic fluorescence upon binding to DNA is proportional to the square of the AT base-pair content. Hydroxystilbamidine is reported to exhibit red fluorescence when bound to calf thymus DNA and T5 DNA, orange fluorescence with Micrococcus lysodeikticus DNA and blue-violet fluorescence on poly(d(AT)). It has been used for the treatment of myeloma, binding selectively to myeloma cells in the bone marrow.

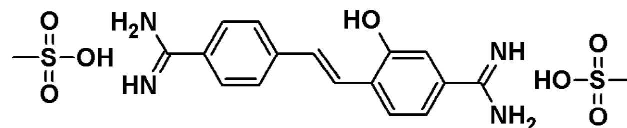

Figure 2.12. The chemical structure of hydroxystilbamidine (Cat# 17514).

LDS 751

LDS 751 (Cat# 17561) is a cell-permeant nucleic acid stain that has been used to discriminate intact nucleated cells from nonnucleated and damaged nucleated cells, as well as to identify distinct cell types in mixed populations of neutrophils, leukocytes and monocytes by flow cytometry. LDS 751, which has its peak excitation at ~543 nm on dsDNA, can be excited by the argon-ion laser at 488 nm and is particularly useful in multicolor analyses due to its long wavelength emission maximum (~712 nm). Binding of LDS 751 to dsDNA results in an ~20-fold fluorescence enhancement.

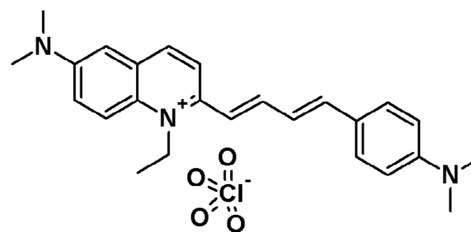

Figure 2.13. The chemical structure of LDS 751 (Cat# 17561).

MycoLight™ Green JJ98 and JJ99

MycoLight™ Green JJ98 (Cat# 24000) and JJ99 (Cat# 24001) are permeable to virtually all cell membranes, including mammalian cells and bacteria. MycoLight™ Green dyes have high molar absorptivities, with extinction coefficients greater than 50,000 $cm^{-1}M^{-1}$ at visible absorption maxima. They have extremely low

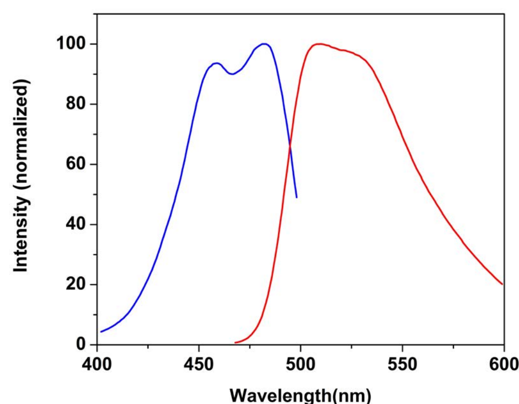

Figure 2.14. The excitation and emission spectra of MycoLight™ JJ98 (Cat# 24000) in the presence of calf thymus DNA.

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intrinsic fluorescence, with quantum yields typically less than 0.01 when not bound to nucleic acids. MycoLight™ Green dyes have great fluorescence enhancement upon binding to DNA. Their quantum yields are typically greater than 0.4 when bound to nucleic acids. MycoLight™ Green stains provide researchers with visible light–excitable dyes for labeling nucleic acids in live cells.

MycoLight™ Green dyes can be excited by both UV and visible light. They can be used to stain RNA and DNA in both live and dead eukaryotic cells, as well as in gram-positive and gram-negative bacteria. MycoLight™ Green JJ98 has a high affinity for DNA and exhibits enhanced fluorescence upon binding to DNA with excitation maximum close to the 488 nm argon laser line and fluorescence emission maximum at ~500 nm. MycoLight™ Green JJ98 is particularly useful as a nuclear counterstain for bacterial assays since it stains both live and dead gram-positive and gram-negative bacteria. It is an excellent replacement for SYTO® 9.

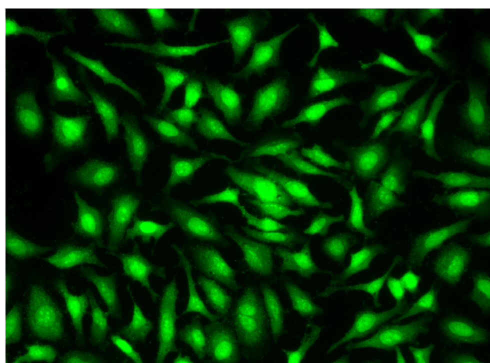

Figure 2.15. Fluorescence image of HeLa cells stained with 5 µM MycoLight™ Green JJ98 (Cat# 24000) for 30 minutes at 37°C.

MycoLight™ Green dyes can stain both DNA and RNA. The fluorescence wavelengths and emission intensities are similar to measurements of DNA or RNA binding in solution. MycoLight™ Green dyes do not exclusively act as nuclear stains in live cells and should not be treated in this regard as DNA-selective compounds, such as DAPI or Hoechst 33258 and Hoechst 33342 dyes, which readily stain cell nuclei at low concentrations in most cells. MycoLight™ Green dye–stained eukaryotic cells will generally show diffuse cytoplasmic staining, as well as nuclear staining. Because MycoLight™ Green dyes are generally cell permeant and contain a net positive charge at neutral pH, they may also stain mitochondria.

Nuclear Green™ LCS1

Our LCS version of Nuclear Green™, Nuclear Orange™, Nuclear Red™ and Nuclear Violet™ nucleic acid stains are cell-permeant cyanine dyes. They cover the full spectrum of visible light, and their distinct colors are particularly useful for multiplexing applications (see Fig 2.17 for their emission spectra). Nuclear Green™ LCS1 (Cat# 17540) is a high-affinity nucleic acid stain that penetrates live cells. It is especially useful for staining both gram-positive and gram-negative bacteria, when an exceptionally bright signal is required. Following brief incubation with Nuclear Green™ LCS1, live cells fluoresce bright green when excited with the 488 nm spectral line of the argon-ion laser or with any other 450–500 nm source. Because Nuclear Green™ LCS1 is essentially nonfluorescent in aqueous medium, no wash steps are required. Unlike DAPI or Hoechst dyes, Nuclear Green™ LCS1 shows little base selectivity.

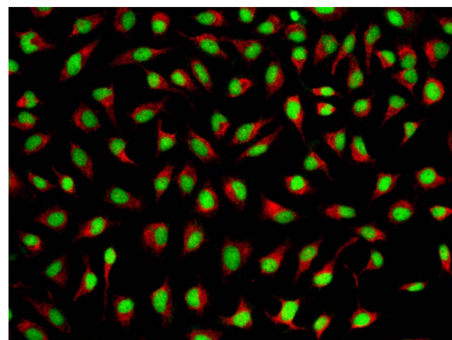

Figure 2.16. Image of live HeLa cells stained with Nuclear Green™ LCS1 (Cat# 17540). The mitochondria of live HeLa cells were stained with red fluorescence Cell Navigator™ Mitochondrion Staining Kit (Cat# 22668).

Nuclear Orange™ LCS1

Nuclear Orange™ LCS1 (Cat# 17541) is a fluorogenic, DNA-selective and cell-permeant dye for analyzing DNA content in live cells. Nuclear Orange™ LCS1 has its orange fluorescence significantly enhanced upon binding DNA. It can be used in fluorescence imaging, microplate and flow cytometry applications. This DNA-binding dye might be used for multicolor analysis of live cells with proper filter sets. Compared with ethidium bromide, Nuclear Orange™ LCS1 has shorter-wavelength emission and its spectra more closely match the rhodamine filter set. In addition, Nuclear Orange™ LCS1 has a much higher molar absorptivity (extinction coefficient) than ethidium bromide and a far greater fluorescence enhancement upon binding DNA, suggesting that it may have a higher sensitivity as a live cell stain or as a nuclear counterstain.

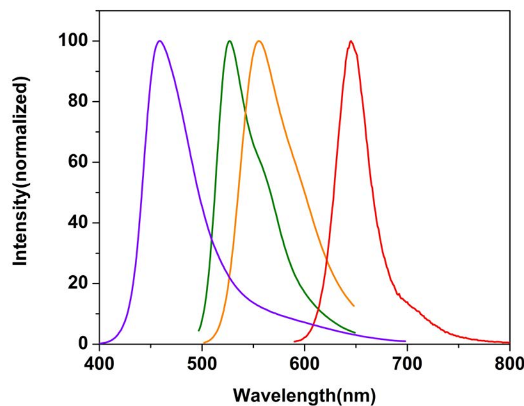

Figure 2.17. The normalized fluorescence spectra of Nuclear Green™ LCS1 (green, Cat# 17540), Nuclear Orange™ LCS1 (orange, Cat# 17541), Nuclear Red™ LCS1 (red, Cat# 17542) and Nuclear Violet™ LCS1 (violet, Cat# 17543).

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Nuclear Red™ LCS1 and LCS2

Nuclear Red™ LCS1 (Cat# 17542) and LCS2 (Cat# 17545) are fluorogenic, DNA-selective and cell-permeant dyes for staining nuclei in live cells. Their red fluorescence is significantly enhanced upon binding DNA. Both Nuclear Red™ LCS1 and LCS2 are well excited by the He-Ne laser used in most flow cytometric applications. It can be used in fluorescence imaging and microplate. These DNA-binding dyes might be used for multicolor analysis of live cells with proper filter sets. Nuclear Red™ LCS2 has longer excitation and emission wavelengths than Nuclear Red™ LCS1, and is compatible with Cy5® filter set.

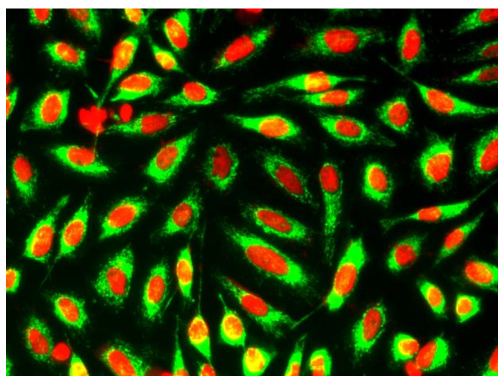

Figure 2.18. Image of live HeLa cells stained with Nuclear Red™ LCS1 (Cat# 17542). The mitochondria of live HeLa cells were stained with green fluorescent Cell Navigator™ Mitochondrion Staining Kit (Cat# 22666).

Nuclear Violet™ LCS1

Nuclear Violet™ LCS1(Cat# 17543) is a fluorogenic, DNA-selective and cell-permeant dye for analyzing DNA content in live cells. Nuclear Violet™ LCS1 has its blue/cyan fluorescence significantly enhanced upon binding DNA. It can be used in fluorescence imaging, microplate and flow cytometry applications. It is well excited by violet laser at 405 nm, and emits blue/cyan fluorescence with an emission maximum at ~440 nm. This DNA-binding dye might be used for multicolor analysis of live cells with the filter sets of Pacific Blue™ and BD Horizon® V450.

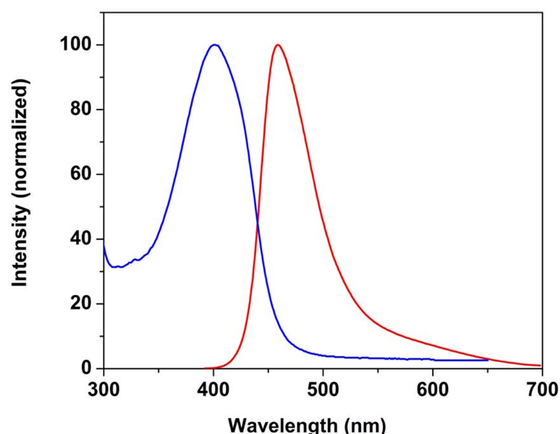

Figure 2.19. The excitation and emission spectra of Nuclear Violet™ LCS1 (Cat# 17543) in the presence of calf thymus DNA.

StrandBrite™ RNA Green

Detecting and imaging RNA molecules in living cells is extremely important for a wide variety of molecular biology procedures including physical transportation, interpretation of genetic information, regulation of gene expression and some essential bio-catalytic roles. The major challenge to stain RNA in living cells is the interferences caused by DNA. In order to address the difficulty, a novel green fluorogenic dye, StrandBrite™ RNA Green, was developed as a RNA-selective probe.

Cell Navigator™ Live Cell RNA Imaging Kit (Cat# 22630) includes StrandBrite™ RNA Green that specifically binds RNA in cells. Compared to commercial SYTO® RNASelect™ dye for RNA staining *in vivo*, StrandBrite™ RNA Green shows brighter signal and much better selectivity to RNA. In addition, this kit can stain RNA in both living cells and fixed cells.

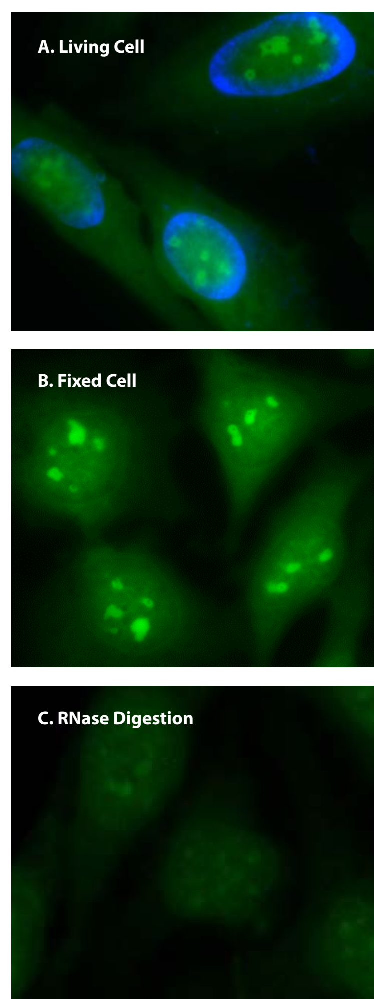

Figure 2.20. Fluorescence images of RNA staining in HeLa cells. (A) Live cells were stained using Cell Navigator™ Live Cell RNA Imaging Kit (Green, Cat# 22630) and counter-stained with Hoechst 33342 (Blue, Cat# 17530). (B) Methanol fixed cells were stained using the same kit. (C) After staining, fixed HeLa cells were incubated with 0.5 mg/mL RNase at 37 ºC for 1 hour. Image of RNase digest test indicates the high selectivity of StrandBrite™ RNA Green. The green fluorescence signal was measured using a fluorescence microscope with a FITC filter.

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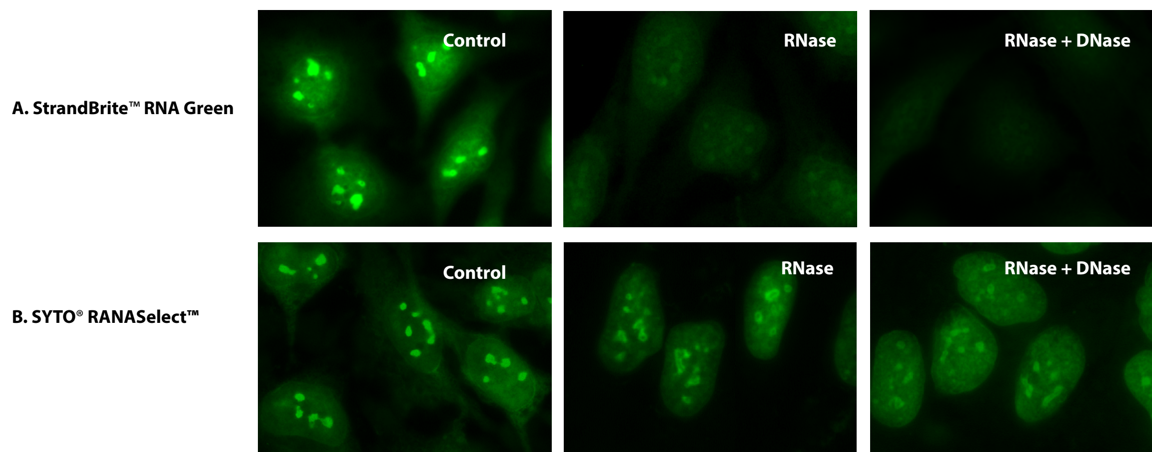

Figure 2.21. RNase and DNase digest tests of HeLa cells stained with (A) StrandBrite™ RNA Green or (B) SYTO® RNASelect™, respectively. Both RNA probes were tested at 1.5 µM concentration. DNase (50 U/mL) and RNase (50 µg/mL) were added into cells. Fluorescence images were taken using a fluorescence microscopy with FITC filter.

Table 2.3 Cell-Permeant DNA Probes & Assay Kits for Staining Nucleic Acids in Live Cells

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22630	Cell Navigator™ Live Cell RNA Imaging Kit *Green Fluorescence*	100 tests	503	511
17510	DAPI Dihydrochloride *UltraPure grade*	10 mg	358	461
17509	DAPI Dilactate	25 mg	358	461
17562	Hexidium Iodide	5 mg	518	600
17520	Hoechst 33258 *UltraPure grade*	100 mg	352	461
17530	Hoechst 33342 *UltraPure grade*	100 mg	350	461
17537	Hoechst 34580 *UltraPure grade*	5 mg	368	437
17514	Hydroxystilbamidine	10 mg	360	625
17561	LDS 751	25 mg	543	712
24000	MycoLight™ Green JJ98 [Replacement for SYTO® 9]	100 µL	484	504
24001	MycoLight™ Green JJ99 [Replacement for SYTO® 9]	100 µL	484	504
17540	Nuclear Green™ LCS1	0.5 mL	503	526
17541	Nuclear Orange™ LCS1	0.5 mL	514	555
17542	Nuclear Red™ LCS1	0.5 mL	622	645
17545	Nuclear Red™ LCS2	0.5 mL	651	681
17543	Nuclear Violet™ LCS1	0.5 mL	401	459
17539	Nuclear Yellow [Hoechst S769121]	25 mg	355	495
17515	Propidium Iodide *UltraPure grade*	25 mg	535	617
17560	PUR-1	1 mg	459	478
17657	StrandBrite™ Green Fluorimetric RNA Quantitation Kit *High Selectivity*	100 tests	508	528
17518	Thiazole Orange *UltraPure grade*	100 mg	512	533

2.3 Cell Cycle Assays

The cell cycle has four sequential phases: G0/G1, S, G2, and M. During a cell's passage through cell cycle, its DNA is duplicated in S (synthesis) phase and distributed equally between two daughter cells in M (mitosis) phase. These two phases are separated by two gap phases: G0/G1 and G2. The two gap phases provide time for the cell to grow and double the mass of their proteins and organelles. They are also used by the cells to monitor internal and external conditions before proceeding with the next phase of cell cycle. The cell's passage through cell cycle is controlled by a host of different regulatory proteins.

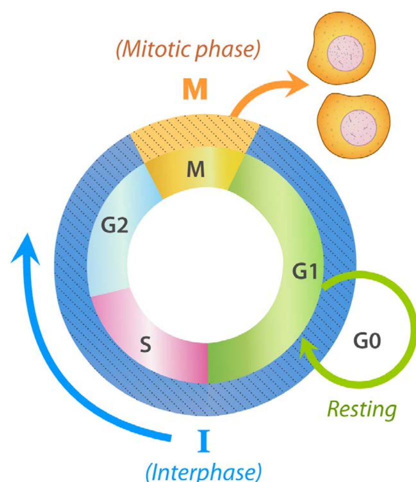

Figure 2.22. Cell division is just one of several stages that a cell goes through during its lifetime. The cell cycle is a repeating series of events that include growth, DNA synthesis, and cell division. The cell cycle in prokaryotes is quite simple: the cell grows, its DNA replicates, and the cell divides. In eukaryotes, the cell cycle is more complicated. The diagram above represents the cell cycle of a eukaryotic cell. As you can see, the eukaryotic cell cycle has several phases. The mitosis phase (M) actually includes both mitosis and cytokinesis. This is when the nucleus and then the cytoplasm divide. The other three phases (G1, S, and G2) are generally grouped together as an interphase. During the interphase, the cell grows, performs routine life processes, and prepares to divide.

AAT Bioquest Cell Meter™ assay kits are a set of tools for monitoring cell viability and proliferation. There are a variety of parameters that can be used for monitoring cell viability and proliferation. In normal cells, DNA density changes depending on whether the cell is growing, dividing, resting or performing its ordinary functions. The progression of the cell cycle is controlled by a complex interplay among various cell cycle regulators. These regulators activate transcription factors, which bind to DNA and turn on or off the production of proteins that result in cell division. Any misstep in this regulatory cascade causes abnormal cell proliferation which underlies many pathological conditions, such as tumor formation. Potential applications for live-cell studies are in the determination of cellular DNA content and cell cycle distribution for detecting variations in growth patterns, for monitoring apoptosis, and for evaluating tumor cell behavior and suppressor gene mechanisms.

Our Cell Meter™ Fluorimetric Cell Cycle Assay Kits (Cat# 22841 & 22842) are designed to monitor cell cycle progression and proliferation by using our proprietary cell cycle dye in permeabilized and fixed cells. The dye passes through a permeabilized membrane and intercalates into cellular DNA. The signal intensity of the cell cycle dye is directly proportional to DNA content. The percentage of cells in a given sample that are in G0/G1, S and G2/M phases, as well as the cells in the sub-G1 phase prior to apoptosis can be monitored with a flow cytometer.

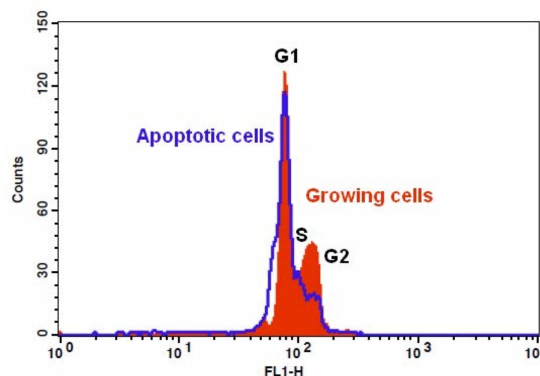

Figure 2.23. Jurkat cells were treated without (red) or with 20 µM camptothecin (blue) in a 37 °C, 5% CO_2 incubator for about 8 hours, and assayed with Cell Meter™ Fluorimetric Cell Cycle Assay kit (Cat# 22841) according to the kit instruction. The fluorescence intensity of Nuclear Green™ LCS1 (Component A) was measured with a FACSCalibur™ flow cytometer using the FL1 channel. In growing Jurkat cells, nuclei stained with Nuclear Green™ LCS1 showed G1, S, and G2 phases (red). In camptothecin treated apoptotic cells (B), the fluorescence intensity of Nuclear Green™ LCS1 was decreased, and both S and G2 phases were diminished.

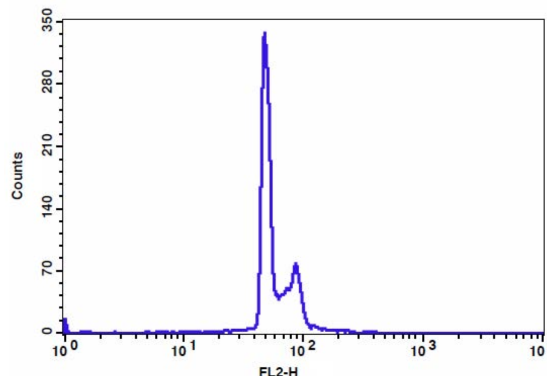

Figure 2.24. Jurkat cells were fixed and dye-loaded with Cell Meter™ Fluorimetric Cell Cycle Assay Kit (Cat# 22842) and RNase A for 30 minutes. The fluorescence intensity of Nuclear Red™ CCS1 (Component A) was measured with the FACSCalibur™ (Becton Dickinson, San Jose, CA) flow cytometer using the FL2 channel.

Table 2.4 Cell Cycle Assays

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22841	Cell Meter™ Fluorimetric Cell Cycle Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 tests	503	526
22842	Cell Meter™ Fluorimetric Cell Cycle Assay Kit *Red Fluorescence Optimized for Flow Cytometry*	100 tests	535	617

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2.4 Cell Apoptosis Assays

Cell Meter™ assay kits are a set of tools for monitoring cell viability. There are a variety of parameters that can be used to monitor cell viability.

Cell Meter™ Nuclear Apoptosis Assay Kit (Cat# 22811) is designed to monitor cell apoptosis through measuring the apoptotic chromatin condensation. The compacted chromatin of apoptotic cells binds higher amounts of nuclear dye compared to the healthy cells. Cell Meter™ Nuclear Apoptosis Assay Kit provides all the essential components with an optimized assay method for the detection of apoptosis in cells with condensed nuclei. This fluorometric assay is based on the detection of the DNA contents in cells using our proprietary non-fluorescent Nuclear Green™ DCS1 that becomes strongly fluorescent upon binding to DNA. In normal cells, Nuclear Green™ DCS1 is not cell permeable. However, apoptotic cells that have compromised plasma membranes or impaired/no cell metabolism are unable to prevent the dye from entering the cell. Once inside the cell, the dyes bind to intracellular DNA producing highly fluorescent complexes which identify the cells as non-viable cells. The staining with Nuclear Green™ DCS1 can be measured using a flow cytometer at Ex/Em = 490 nm/520 nm (FL1 channel) or fluorescence microscope (FITC filter set). The kit can be used with our other apoptosis reagents, such as our Cell Meter™ NIR Mitochondria Membrane Potential Detection Kit (Cat# 22802), for multi-parametric study of cell viability and apoptosis. The kit is optimized for screening apoptosis activators and inhibitors.

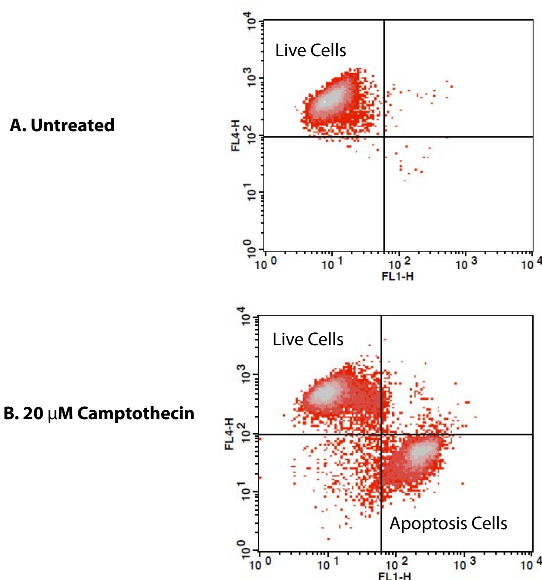

Figure 2.25. The increase in fluorescence intensity of Nuclear Green™ DCS1 (Cat# 17550) with the addition of camptothecin in Jurkat cells. Jurkat cells were treated overnight without (A) or with 20 µM camptothecin (B) in a 37 °C, 5% CO_2 incubator, and then dye loaded with Nuclear Green™ DCS1 for 60 minutes. At the end of 15 minutes of Nuclear Green™ DCS1 dye loading, MitoLite™ NIR (Cat # 22802) was added for multicolor analysis. The fluorescence intensities of Nuclear Green™ DCS1 and MitoLite™ NIR were measured with a FACSCalibur (Becton Dickinson) flow cytometer using FL1 channel (Nuclear Green™ DCS1) and FL4 channel (MitoLite™ NIR).

Annexins are a family of proteins that bind to phospholipid membranes in the presence of calcium. Annexin V is a valuable tool for studying cell apoptosis. It is used as a probe to detect cells which have expressed phosphatidylserine on the cell surface, a feature found in apoptosis as well as other forms of cell death. There are a variety of parameters that can be used for monitoring cell viability. Annexin V-dye conjugates are widely used to monitor cell apoptosis through measuring the translocation of phosphatidylserine (PS). In apoptosis, PS is transferred to the outer leaflet of the plasma membrane. The appearance of phosphatidylserine on the cell surface is a universal indicator of the initial/intermediate stages of cell apoptosis and can be detected before morphological changes can be observed.

Annexin V conjugates bind to PS on apoptotic cell surfaces in the presence of Ca^{2+}, it also can pass through cell membranes of necrosis or dead cells and bind to PS in the interior of cells. Therefore, we recommend using a cell-impermeable nuclear stain in combination with annexin V conjugate to distinguish dead cells from apoptotic cells. Annexin V-iFluor™ 555 Conjugate (Cat# 20072) has spectral properties similar to Alexa Fluor® 555 and Cy3®.

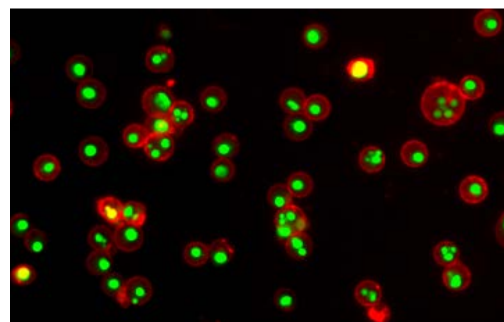

Figure 2.26. Image of apoptotic Jurkat cells. Cells were treated with staurosporine (1 µM) for 4 hours, and then stained with Annexin V-iFluor™ 555 Conjugate (red, Cat# 20072), and nuclei were stained with Nuclear Green™ DCS1 (green, Cat# 17550).

Cell Meter™ Annexin V Binding Apoptosis Assay Kit (Cat# 22828) uses our proprietary blue fluorescent Annexin-V mFluor™ Violet 450 PS sensor that specifically binds PS with blue fluorescence. The stain has the spectral properties similar to those of Pacific Blue™ at Ex/Em= ~405/450 nm (Pacific Blue™ is the trademark of Invitrogen). The blue fluorescence stain is well excited with the violet laser at 405 nm, and emits intense blue fluorescence at ~450 nm.

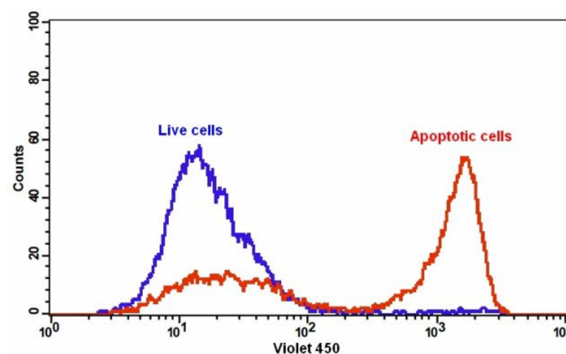

Figure 2.27. The detection of binding activity of Annexin V-mFluor Violet™ 450 and phosphatidylserine in Jurkat cells Cell Meter™ Annexin V Binding Apoptosis Assay Kit (Cat# 22828). Jurkat cells were treated without (Blue) or with 20 µM camptothecin (Red) in a 37 °C, 5% CO_2 incubator for 4-5 hours, and then dye loaded with Annexin V-mFluor Violet™ 450 for 30 minutes. The fluorescence intensity of Annexin V-mFluor Violet™ 450 was measured with a FACSCalibur (Becton Dickinson) flow cytometer using violet laser at 450 nm channel.

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Cell Meter™ Phosphatidylserine Apoptosis Assay Kit (Cat# 22831) uses our proprietary green fluorescent Apopxin™ PS sensor that specifically binds PS with affinity much higher than Annexin V (K_d < 10 nM). The PS sensor used in this kit has green fluorescence upon binding to membrane PS. The stain has the spectral properties almost identical to those of FITC with much higher photostability, making it convenient to be used with the common fluorescence instruments equipped with the light sources and filters for FITC, the most common fluorophore. This kit is optimized for flow cytometry applications while Cell Meter™ Phosphatidylserine Apoptosis Assay Kit (Cat# 22791) is optimized to be used with a fluorescence microplate reader or a fluorescence microscope. This kit is a convenient alternative to Annexin V FITC conjugate.

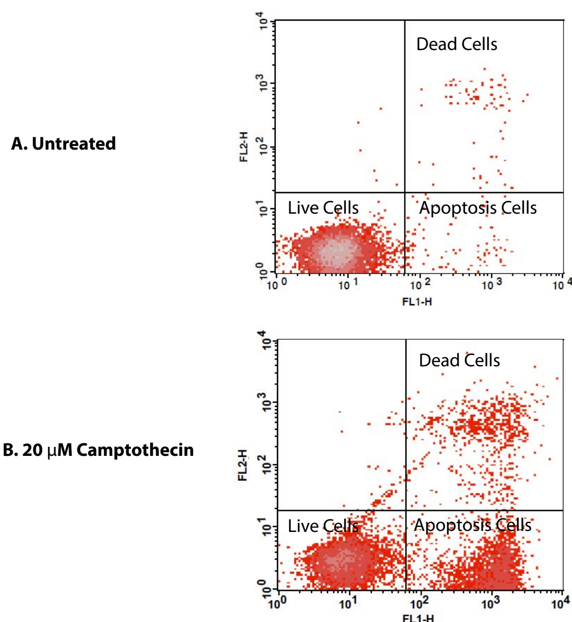

Figure 2.28. The detection of binding activity of Apopxin™ Green and phosphatidylserine in Jurkat cells using Cell Meter™ Phosphatidylserine Apoptosis Assay Kit (Cat# 22831). Jurkat cells were treated without (A) or with 20 µM camptothecin (B) in a 37 °C, 5% CO_2 incubator for 5 hours, and then dye loaded with Apopxin™ Green and propidium iodide for 30 minutes. The fluorescence intensity of Apopxin™ Green was measured with a FACSCalibur (Becton Dickinson) flow cytometer using the FL1 channel and the fluorescence intensity of propidium iodide was measured using the FL2 channel.

Cell Meter™ Phosphatidylserine Apoptosis Assay Kit (Cat# 22832) uses our proprietary red fluorescent Apopxin™ PS sensor that specially binds PS with affinity much higher than Annexin V (K_d < 10 nM). The PS sensor used in this kit has red fluorescence upon binding to membrane PS. The stain has the spectral properties almost identical to those of Cy5® or Alexa Fluor® 647, making it convenient to be used with the common fluorescence instruments equipped with the light sources and filters for Cy5® or Alexa Fluor® 647. Due to its highly enhanced affinity to PS, this kit is more robust than the other commercial Annexin V based apoptosis kits and can be also used with a flow cytometry platform.

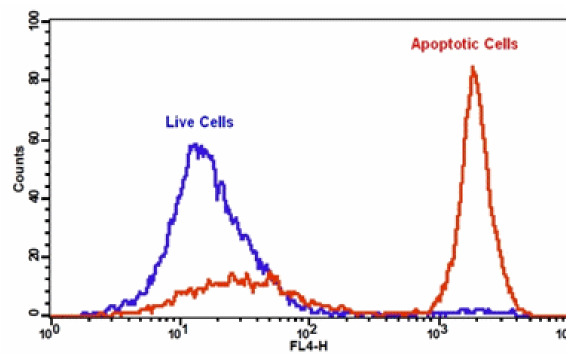

Figure 2.29. Detection of phosphatidylserine binding activity in Jurkat cells using Cell Meter™ Phosphatidylserine Apoptosis Assay Kit (Cat# 22832) . Jurkat cells were treated without (Blue) or with 20 µM camptothecin (red) in a 37 °C, 5% CO_2 incubator for 4-5 hours, and then loaded with Apopxin™ Deep Red for 30 minutes. The fluorescence intensity of Apopxin™ Deep Red was measured with a FACSCalibur (Becton Dikinson) flow cytometer in FL4 channel.

Table 2.5 Cell Apoptosis Assays

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
20072	Annexin V-iFluor™ 555 Conjugate	100 tests	556	574
22828	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Blue Fluorescence Excited at 405 nm*	100 tests	405	450
22829	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Green Fluorescence Excited at 405 nm*	100 tests	414	508
22830	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Orange Fluorescence Excited at 405 nm*	100 tests	424	560
22837	Cell Meter™ APC-Annexin V Binding Apoptosis Assay Kit *Optimized for Flow Cytometry*	100 tests	651	662
22811	Cell Meter™ Nuclear Apoptosis Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 tests	503	526
22838	Cell Meter™ PE-Annexin V Binding Apoptosis Assay Kit *Optimized for Flow Cytometry*	100 tests	565	575
22935	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Blue Fluorescence Excited at 405 nm*	100 tests	405	450
22793	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Deep Red Fluorescence Optimized for Microplate Readers*	100 tests	649	660
22832	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Deep Red Fluorescence Optimized for Flow Cytometry*	100 tests	649	660
22791	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Green Fluorescence Optimized for Microplate Readers*	100 tests	498	520
22831	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 tests	498	520

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Quantifying Nucleic Acids in Solutions

3

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The scope of nucleic acid-based assays continues to expand. There is no consensus on the choice of a method for nucleic acid detection. Key factors governing detection method choice include stability, sensitivity, speed and convenience, and the overall cost for the detection reagents and detection system. The reasons for ultrasensitive analysis in nucleic acid include analysis of genetic material from single cells and from rare cells, such as trophoblasts in maternal circulation, and single-copy gene detection. In addition, better detection methods may provide a route to assays that require fewer amplification cycles or eliminate the multicycle protocols for nucleic acid amplification reactions (e.g., PCR). Although many detection technologies, such as those based on chemiluminescence, may play an important role in the future, at present fluorescence is still the most widely used detection technique in analyzing nucleic acids.

Helixyte™ Green dsDNA Quantifying Reagent (Cat# 17597) and StrandBrite™ Green RNA Quantifying Reagent (Cat# 17611) are optimized for double-stranded DNA and single-stranded RNA respectively. They have a high affinity for nucleic acids and an extremely large fluorescence enhancement upon binding nucleic acids, making it possible to directly detect minute amounts of nucleic acids in complex solutions within minutes, usually without interference from other biomolecules. These reagents and quantitation assays provide the following advantages:

- ***High Sensitivity:*** The Helixyte™ Green and StrandBrite™–based fluorescence assays are up to 10,000-fold more sensitive than UV absorbance measurements.
- ***Great Selectivity:*** Unlike measurements of UV absorbance, these assays are not affected by the presence of proteins, free nucleotides or very short oligonucleotides, making quantitation of intact oligonucleotides and nucleic acids much more accurate in complex mixtures, such as serum or whole blood.
- ***Great Convenience:*** These assays have a very simple protocol that requires no separation steps, making them ideal for automated, high-throughput measurements.
- ***Broad Dynamic Range:*** Quantitation is accurate over four orders of magnitude for the Helixyte™ Green-based assays. The StrandBrite™–based fluorescence assays is accurate over three orders of magnitude.

3.1 dsDNA Quantification Assay

Helixyte™ Green Fluorimetric dsDNA Assay Kits (Cat# 17650 & 17651) simplify DNA quantification without sacrificing sensitivity. The assay provide a linear detection range from 0.2 ng to 1000 ng double-stranded DNA (dsDNA). This high-sensitive DNA assay is ideal for quantifying PCR products, viral DNA, DNA fragments for subcloning and other applications requiring small amounts of DNA. Helixyte™ Green Fluorimetric dsDNA Assay Kits are highly selective for dsDNA over RNA and other common contaminants, including free nucleotides, salts, solvents and proteins. The Helixyte™ Green assay is an excellent replacement for PicoGreen®-based DNA assays.

Helixyte™ Green dsDNA Quantitation Kit (Cat# 17652) uses Helixyte Green™ BDR (broad range) as a sensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in solution. Helixyte Green™ BDR has recently been used to quantitate PCR amplification yields in direct cycle sequencing of PCR products. Helixyte Green™ BDR is highly selective for dsDNA over ssDNA, RNA, and free nucleotides, and it is accurate for initial sample concentrations from 25 ng/mL to 50 µg/mL, which is comparable with Qubit® dsDNA BR Reagent.

Helixyte™ Green dsDNA Quantitation Kit is optimized for fluorescence microplate reader, and can be readily adapted to be used in spectrofluorometer such as Qubit® Fluorometer. It is ideal for accurately measuring DNA from many sources, including genomic DNA, viral DNA, mini prep DNA, or PCR.

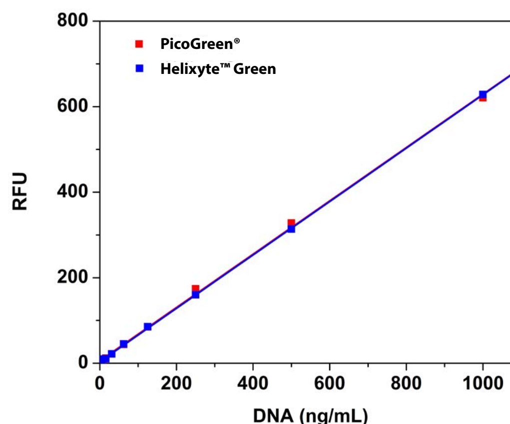

Figure 3.1. The quantification of calf thymus DNA with Helixyte™ Green vs. PicoGreen®. Helixyte™ Green and PicoGreen® have almost identical performance.

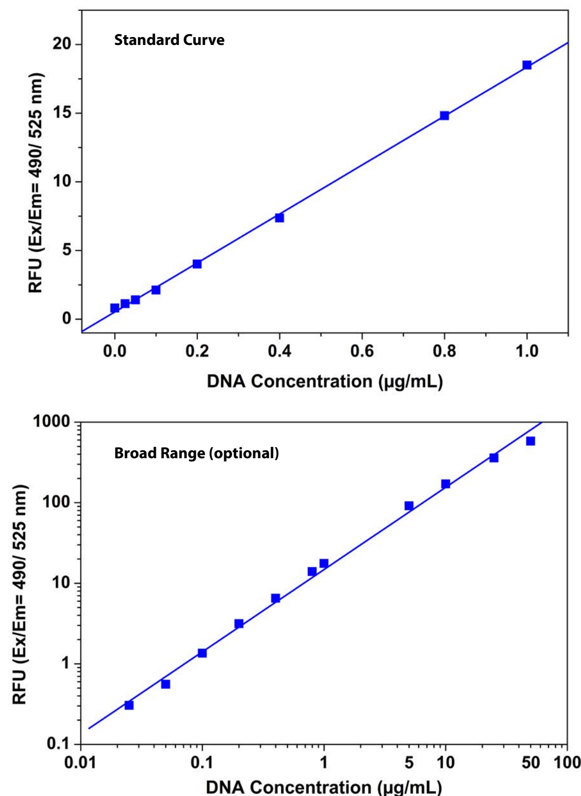

Figure 3.2. DNA dose response was obtained with Helixyte™ Green dsDNA Quantitation Kit (Cat# 17652). DNA standards in the concentration range from 25 ng/mL to 50 µg/mL were stained on a solid black 96-well plate and measured using a Gemini microplate reader (Molecular Devices).

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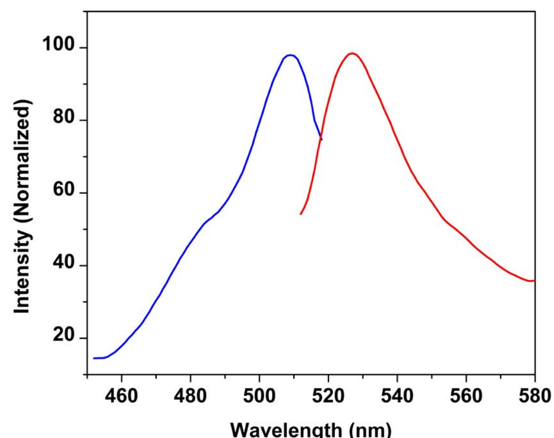

Figure 3.3. The excitation and emission spectra of Helixyte Green™ BDR in the presence of calf thymus DNA.

Helixyte™ Green dsDNA Quantifying Reagent (Cat# 17597) can accurately quantify as little as 100 pg/mL dsDNA using a fluorometer or 300 pg/mL dsDNA (typically 50 pg in a 200 µL volume) using a fluorescence microplate reader. The Helixyte™ Green dsDNA quantitation assay is 10,000 times more sensitive than conventional UV absorbance measurements at 260 nm and at least 400 times more sensitive than the Hoechst 33258 dye–based assay. Helixyte™ Green dsDNA Quantifying Reagent shows a >1000-fold fluorescence enhancement upon binding to dsDNA, and much less fluorescence enhancement upon binding to single-stranded DNA (ssDNA) or RNA, making it possible to quantify dsDNA in the presence of equimolar amounts of ssDNA, RNA or proteins. In comparison to other common DNA dyes, such as Hoechst 33258 dye, which show significant base selectivity, Helixyte™ Green dsDNA Quantifying Reagent shows little if any AT- or GC-selectivity, enabling accurate DNA quantification.

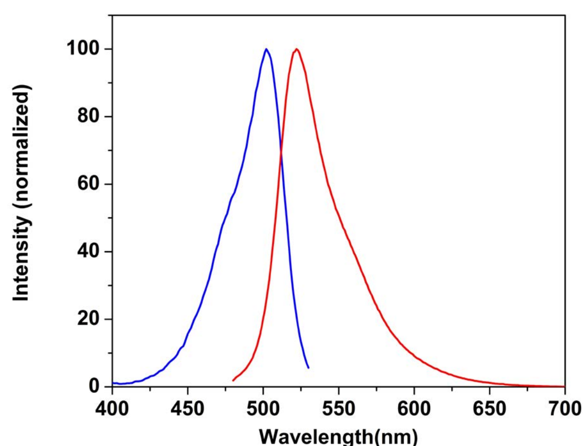

Figure 3.4. The excitation and emission spectra of Helixyte™ Green in the presence of calf thymus DNA.

The protocol of the Helixyte™ Green dsDNA quantitation assay is in a simple mix and read format, i.e., the dye is simply added to the sample and incubated for five minutes, then the fluorescence is measured. In addition, the fluorescence signal from binding of the Helixyte™ Green reagent to dsDNA is linear over at least four orders of magnitude with a single dye concentration, whereas assays using ethidium bromide or Hoechst 33258 dye exhibit a much more limited linear range. The linearity is maintained even in the presence of several interfering compounds commonly found in nucleic acid preparations, including salts, urea, ethanol, chloroform, detergents, proteins and agarose. The Helixyte™ Green reagent can be excited at 488 nm with an argon-ion laser, and is a superior nucleic acid stain for analysis of single DNA molecules using a flow cytometer.

The Helixyte™ Green assay is useful for quantifying DNA templates for PCR, labeling reactions, electrophoretic mobility-shift (band-shift) assays, DNA-footprinting assays and filter-binding assays, and for measuring yields from PCR reactions, DNA minipreps and maxipreps, cDNA synthesis and nuclease protection assays. The simplicity and selectivity of Helixyte™ Green also make it ideal for high-throughput quantification assays used in forensic and genomics research.

Hoechst 33258 (Cat# 17520) has been extensively used to quantify dsDNA in solution. Hoechst 33258 shows a fluorescence increase upon binding nucleic acids and a preference for binding to AT regions. Hoechst 33258 is selective for dsDNA over RNA in high-salt buffers and for dsDNA over ssDNA in low-salt buffers. Hoechst 33258 can quantitatively detect from 10 ng/mL to ~10 µg/mL dsDNA when two different dye concentrations are used. While this assay uses principles that are similar to other fluorescent assays, newer dyes, such as the Helixyte™ Green reagent, provide much higher sensitivity, better selectivity and a broader dynamic range with a single dye concentration.

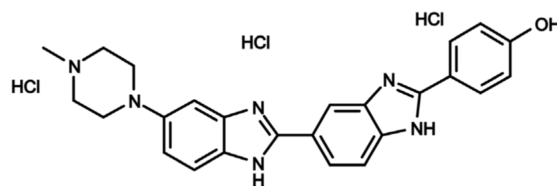

Figure 3.5. The chemical structure of Hoechst 33258 (Cat# 17520).

Table 3.1 dsDNA Quantification Reagents and Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
17597	Helixyte™ Green dsDNA Quantifying Reagent	1 mL	501	520
17652	Helixyte™ Green dsDNA Quantitation Kit *Broad Detection Range*	200 tests	501	520
17651	Helixyte™ Green Fluorimetric dsDNA Quantitation Kit *High Sensitivity*	200 tests	501	520
17650	Helixyte™ Green Fluorimetric dsDNA Quantitation Kit *Optimized for Microplate Readers*	200 tests	501	520
17520	Hoechst 33258 *UltraPure Grade*	100 mg	352	461
17530	Hoechst 33342 *UltraPure grade*	100 mg	350	461
17537	Hoechst 34580 *UltraPure grade*	5 mg	368	437

3.2 RNA Quantification Assay

The major challenge to analyze RNA in live cells is the interferences caused by DNA. To address these difficulties, AAT Bioquest has developed an excellent RNA-selective probe that generates significantly enhanced green fluorescence upon binding to RNA. It has been successfully used for flow cytometric analysis of live cells. It has the excitation/emission of 490/540 nm. In the DNase digest test, no significant change of fluorescence intensity in fixed cells stained with the RNA-selective probe was observed. In contrast, after RNase digestion, the initial fluorescence signal decreased immediately. These results indicate that initial fluorescence signal was generated from the specific interaction of the RNA-selective probe with RNA in cells. Short exposure of live cells to antinomycin D did cause inhibition of RNA synthesis during 6 hours after drug removal in a dose-dependent manner. These data demonstrate that the RNA-selective probe is a sensitive RNA-selective dye for staining nucleolar RNA in live and fixed cells. The RNA-selective probe has less DNA interferences than the commonly used SYTO® RNASelect™ dye. It is a highly RNA-selective fluorescent probe. Due to its excellent cell permeability and spectral properties, it has been successfully used for flow cytometric RNA analysis and fluorescence microscope in live cells. It can be well excited with the 488 nm blue laser and monitored in FITC channel. The RNA-selective probe provides a valuable method for identifying and labeling cells with a single incubation step and can discriminate RNA from DNA with better selectivity than the commonly used SYTO® RNASelect™.

StrandBrite™ Green Fluorimetric RNA Quantitation Kits (Cat# 17655 & 17656) provide a homogeneous assay for quantifying RNA in the presence of DNA. This RNA assay exhibits a linear detection range from 5 ng to 100 ng RNA. Assay linearity is maintained even in the presence of several interfering compounds commonly found in nucleic acid preparations, including salts, urea, ethanol, chloroform, detergents, proteins and agaroses. Its relatively high selectivity for RNA over dsDNA enables accurate RNA quantification in the presence of DNA and other common contaminants, including free nucleotides, salts, solvents and proteins, making this assay ideal for measuring samples for microarray, RT-PCR and northern blot procedures.

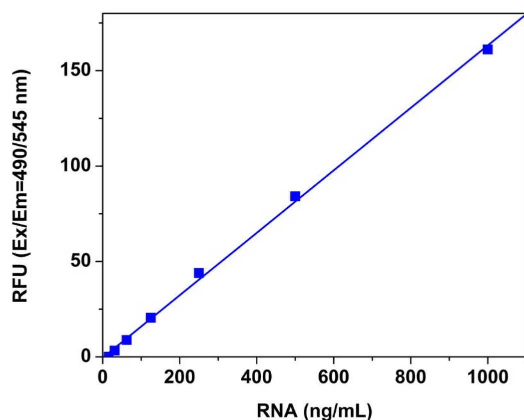

Figure 3.6. The quantification of RNA with StrandBrite™ Green Fluorimetric RNA Quantitation Kit (Cat# 17655).

StrandBrite™ Green Fluorimetric RNA Quantitation Kit (Cat# 17657) includes StrandBrite™ RNA Green with an optimized and robust protocol. It provides a convenient and sensitive method for quantifying RNA in solutions.

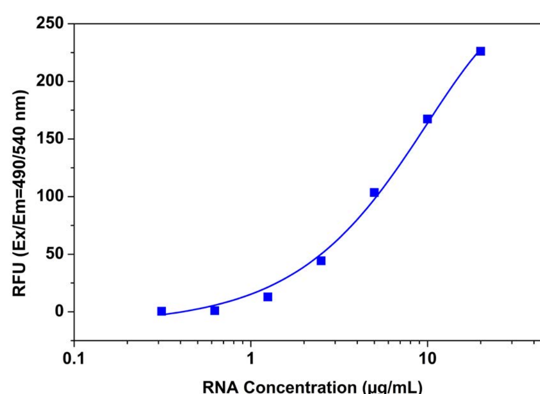

Figure 3.7. RNA dose response measured with StrandBrite™ Green Fluorimetric RNA Quantitation Kit (Cat# 17657) in a solid black 96-well microplate using a Gemini microplate reader (Molecular Devices).

StrandBrite™ Green RNA Quantitation Reagent (Cat# 17610) allows detection as little as 10 ng/mL RNA using a standard fluorometer, fluorescence microplate reader or filter-based fluorometer with standard fluorescein excitation and emission settings. The sensitivity is at least 20-fold better than that achieved with ethidium bromide and at least 100-fold better than that achieved using conventional absorbance measurements at 260 nm. Unlike UV absorbance measurements at 260 nm, StrandBrite™ Green RNA Quantitation Reagent does not detect significant sample contamination caused by free nucleotides. Thus, the StrandBrite™ Green RNA Quantitation Reagent more accurately measures the amount of intact RNA polymers in potentially degraded samples.

Table 3.2 RNA Quantification Reagent and Assay Kits

Cat #	Product Name	Size	Ex (nm)	Em (nm)
17656	StrandBrite™ Green Fluorimetric RNA Quantification Kit	100 tests	500	525
17657	StrandBrite™ Green Fluorimetric RNA Quantification Kit *High Selectivity*	100 tests	508	528
17655	StrandBrite™ Green Fluorimetric RNA Quantification Kit *Optimized for Microplate Readers	1000 tests	485	549
17610	StrandBrite™ Green RNA Quantifying Reagent	1 mL	485	549

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3.3 Real-Time Quantitative PCR

Measurements of PCR products can be taken during the linear portion of the amplification reactions, allowing accurate quantification of templates. Several methods exist for real-time quantification of PCR products, including fluorescence resonance energy transfer techniques using fluorescently labeled primers or molecular beacon (for the detailed information, please request our mini-catalog: Oligonucleotides Labeling Reagents). Identification of PCR products during the reaction can also be monitored using Cyber Green™. In addition, individual DNA molecules have been detected with on-line capillary PCR coupled with laser-induced fluorescence detection by adding Cyber Green™ stain to the reaction mixture.

Cyber Green™ binds preferentially to dsDNA, allowing accurate quantification of double-stranded products in the presence of single-stranded oligonucleotide primers. Cyber Green™ displays high stability in temperature extremes required for PCR reactions and does not interfere with Taq polymerase. Improved specificity for quantitating desired products can be achieved by using Cyber Green™ after the assay to measure the melting temperature of the products. Double-stranded DNA with no base mismatches will show a higher melting temperature than the nonspecific templates that contain mismatches. Real-time quantitative PCR experiments can be carried out by using instruments specialized for the application or by quantifying amplification products manually at different time points. Real-time quantitative PCR with Cyber Green™ has been used to develop reliable and simple assays for detecting genetic mutations, including duplications and deletions in mosquito drug-resistance genes, chromosomal translocations in human disease genes, and base substitutions. It has also been used for the unequivocal identification of viral, bacterial or fungal pathogens. In addition, this method has been used successfully for quantitative reverse-transcription PCR.

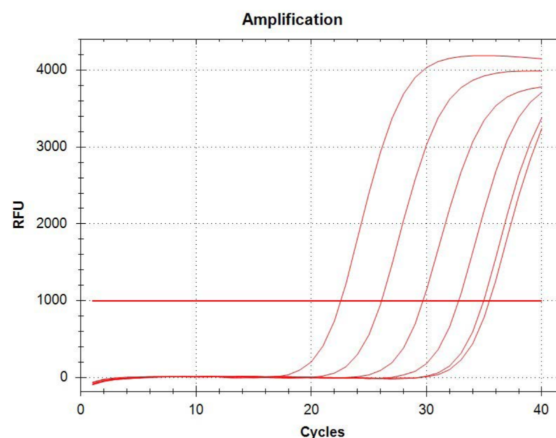

Figure 3.9. Amplification curves of the real-time quantitative PCR of 10-fold serial dilutions (from $3X10^5$ to 3 copies) of KIR genes (Killer Cell Immunoglobulin-like Receptor) using Cyber Green™ (Cat# 17591).

Table 3.3 Fluorescent Probes for Quantifying DNA Samples in Solutions

Cat #	Product Name	Size	Ex (nm)	Em (nm)
17004	AA-dUTP [Aminoallyl dUTP sodium salt] *4 mM in Tris Buffer (pH 7.5)*	1 µmole	N/A	N/A
17021	AA-UTP [Aminoallyl UTP sodium salt] *4 mM in TE Buffer*	250 µL	N/A	N/A
17016	Biotin-11-dUTP *1 mM in Tris Buffer (pH 7.5)*	25 nmoles	N/A	N/A
17017	Biotin-16-dUTP *1 mM in Tris Buffer (pH 7.5)*	25 nmoles	N/A	N/A
17018	Biotin-20-dUTP *1 mM in Tris Buffer (pH 7.5)*	25 nmoles	N/A	N/A
17591	Cyber Green™ *20X Aqueous PCR Solution*	5x1 mL	497	520
17590	Cyber Green™ *10,000X Aqueous PCR Solution*	1 mL	497	520
17024	DEAC-dUTP *1 mM in Tris Buffer (pH 7.5)*	25 nmoles	420	460
17022	Fluorescein-dUTP *1 mM in Tris Buffer (pH 7.5)*	25 nmoles	492	515
17011	mFluor™ Violet 450-dUTP *1 mM in Tris Buffer (pH 7.5)*	25 nmoles	405	450
395	6-ROX Glycine *25 uM fluorescence reference solution for PCR reactions*	5 mL	575	602
17023	Tetramethylrhodamine-dUTP *1 mM in Tris Buffer (pH 7.5)*	25 nmoles	546	564
17006	TF1-dUTP *1 mM in Tris Buffer (pH 7.5)*	25 nmoles	420	460
17007	TF2-dUTP *1 mM in Tris Buffer (pH 7.5)*	25 nmoles	499	522
17008	TF3-dUTP *1 mM in Tris Buffer (pH 7.5)*	25 nmoles	554	578
17009	TF4-dUTP *1 mM in Tris Buffer (pH 7.5)*	25 nmoles	588	610
17010	TF5-dUTP *1 mM in Tris Buffer (pH 7.5)*	25 nmoles	656	670

4 Detecting Nucleic Acids in Gels

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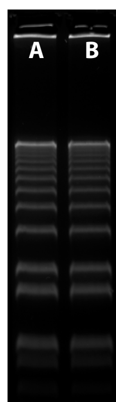
Ethidium bromide (EtBr) is commonly used to detect nucleic acids in gels. The detection typically involves a gel containing nucleic acids placed on or under a UV lamp. Since ultraviolet light is harmful to eyes and skin, gels stained with ethidium bromide are usually viewed indirectly using an enclosed camera, with the fluorescent images recorded as photographs. When direct viewing is needed, the viewer's eyes and exposed skin should be protected. Due to the safety concerns with ethidium bromide, there are increasing alternatives to ethidium bromide which are advertised as being less dangerous by some commercial vendors. However, any compounds that interact with nucleic acid should be handled with great caution, and the waste should be handled by properly trained personnel since they are inherently toxic. For example, SYBR® Green I is less mutagenic than ethidium bromide by the Ames test with liver extract. However, SYBR® Green I was actually found to be more mutagenic than EtBr to the bacterial cells exposed to UV. This may be the case for other "safer" dyes too.

4.1 Cyber Green™ Nucleic Acid Gel Stain & Gelite™ Green Gel Staining Kit

Cyber Green™ Nucleic Acid Gel Stain (Cat# 17590) is a sensitive fluorescent nucleic dye for detecting nucleic acids in agarose and polyacrylamide gels. The gel stain exhibits an exceptional affinity to DNA and a large fluorescence enhancement upon binding DNA, at least one order of magnitude greater than that of ethidium bromide when detected by photography. With a standard 300 nm UV transilluminator and photographic detection, as little as 60 pg dsDNA per band can be detected with Cyber Green™ gel stain (Figure 4.1). Cyber Green™ Nucleic Acid Gel Stain is nearly two orders of magnitude more sensitive than ethidium bromide for staining oligonucleotides in gels.



A: Cyber Green™

B: SYBR® Green

Figure 4.1. DNA molecular weight ladders stained with Cyber Green™ (Cat# 17590) and SYBR® Green I Nucleic Acid Gel Stain.

Cyber Green™ Nucleic Acid Gel Stain is not much more sensitive than ethidium bromide for staining RNA. It has a much greater fluorescence enhancement when bound to dsDNA and oligonucleotides than when bound to RNA. The selectivity makes Cyber Green™ Nucleic Acid Gel Stain ideal for applications in which DNA samples may be contaminated with RNAs, such as when visualizing DNA fragmentation ladders from apoptotic cells. Cyber Green™ gel stain shows very low background fluorescence in the gel, making it the preferred dye for some laser-scanning instruments, in which background fluorescence can produce unacceptable noise levels.

Cyber Green™ gel stain has a UV-excitation peak of ~250 nm (Figure 4.2). Thus, higher sensitivity can be achieved with Cyber Green™ stain using 254 nm transillumination, as compared with the more common 300 nm transillumination. However, the visible excitation peak of Cyber Green™ dye–stained nucleic acids near 497 nm is very close to the principal emission lines of many laser-scanning instruments. Because nucleic acid–bound Cyber Green™ gel stain exhibits spectral characteristics (excitation/emission maxima ~497/520 nm) very similar to those of fluorescein, it is compatible with most common filter sets used in laser scanners. Cyber Green™ gel stain is very easy to use with no destaining step required prior to photography. Typical staining concentrations of Cyber Green™ gel stain does not significantly inhibit the ability of several restriction endonucleases to cleave DNA. This property makes staining with Cyber Green™ dye compatible with in-gel subcloning protocols. Cyber Green™ stain is also easily removed from dsDNA by simple ethanol precipitation.

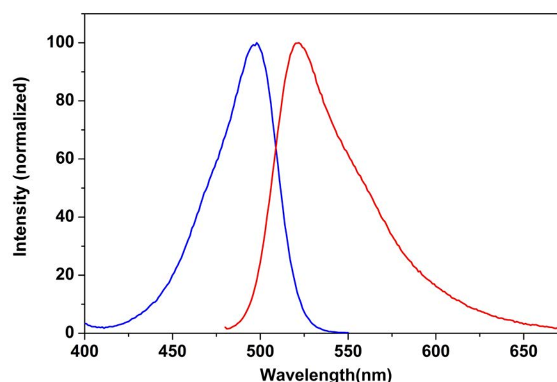


Figure 4.2. The excitation and emission spectra of Cyber Green™ Nucleic Acid Gel Stain (Cat# 17590) in the presence of calf thymus DNA.

The ultrasensitivity of Cyber Green™ gel stain makes it useful for detecting the products of DNA and RNA amplification reactions by gel electrophoresis, restriction mapping small amounts of DNA and detecting the products of bandshift and nuclease-protection assays. PCR amplification products that are at the limit of detection using ethidium bromide are easily detected using Cyber Green™ dye. Reverse-transcription PCR (RT-PCR) reaction products have been detected with high sensitivity following gel electrophoresis and staining with Cyber Green™ gel stain, allowing the cycle number to be lowered, which reduces heteroduplex formation during amplification

Cyber Green™ Nucleic Acid Gel Stain may enable researchers to eliminate silver staining and radioactivity from their protocols. Cyber Green™ gel stain was shown to be as sensitive as silver staining, but more rapid, less laborious and less expensive than a radioactive method. Cyber Green™ gel stain can be used for replacing conventional silver staining techniques for the routine

Typical Staining Protocol for Cyber Green™ Gel Stain:

1. Post-staining protocol

1.1 Run gels based on your standard protocol.
1.2 Make 1X Cyber Green™ working solution by diluting the 10,000X stock reagent into PH 7.5 - 8 buffer (e.g., TAE, TBE or TE preferably pH 8.0).
Note: Staining solutions prepared in water are less stable than those prepared in buffer and must be used within 24 hours to ensure maximal staining sensitivity. In addition, staining solutions prepared in buffers with pH below 7.5 or above 8.0 are less stable and show reduced staining efficacy.
1.3 Place the gel in a suitable polypropylene container. Gently add a sufficient amount of the 1X staining solution to submerge the gel.
1.4 Agitate the gel gently at room temperature for ~30 minutes, protecting from light.
Note: The staining solution may be stored in the dark (preferably refrigerated) for a week and reused up to 2-3 times.
1.5 Image the stained gel with a 254 nm transilluminator, or a laser-based gel scanner using a long path green filter, such as a SYBR® filter or GelStar® filter.

2. Pre-casting protocol

2.1 Prepare agarose gel solution using your standard protocol.
2.2 Dilute the 10,000X Cyber Green™ stock reagent into the gel solution at 1:10,000 just prior to pouring the gel and mix thoroughly.
2.3 Run gels based on your standard protocol.
2.4 Image the stained gel with a 254 nm transilluminator, or a laser-based gel scanner using a long path green filter such as a SYBR® filter or GelStar® filter.

3. DNA-staining before electrophoresis

3.1 Incubate DNA with a 1:10,000 dilution of the dye (in TE, TBE, or TAE) for at least 15 minutes prior to electrophoresis.
3.2 Run gels based on your standard protocol.
3.3 Image the stained gel with a 254 nm transilluminator, or a laser-based gel scanner using a long path green filter, such as a SYBR® filter or GelStar® filter.

identity testing in some forensics laboratories. Our Gelite™ Green Nucleic Acid Gel Staining Kit (Cat# 17589) includes our Cyber Green™ nucleic acid gel stain as a key component. The kit provides an optimized and robust protocol for staining nucleic acid samples in gels.

4.2 Cyber Orange™ Nucleic Acid Gel Stain & Gelite™ Orange Gel Staining Kit

Cyber Orange™ Nucleic Acid Gel Stain (Cat# 17595) is an extremely sensitive nucleic acid dye for detecting DNA or RNA in gels using a standard 300 nm UV transilluminator and Polaroid 667 black-and-white print film. As with Cyber Green™ gel stain, the remarkable sensitivity of Cyber Orange™ can be attributed to a combination of unique dye characteristics. Because the nucleic acid–bound Cyber Orange™ gel stain exhibits excitation maxima at both ~495 nm and ~300 nm (the emission maximum is ~537 nm, Figure 4.3), it is compatible with a wide variety of instrumentation, ranging from UV transilluminators and Epi-blue light transilluminators, to mercury-arc lamp and argon-ion laser–based gel scanners. Short-wavelength (254 nm) epi-illumination is not required to obtain high sensitivity with Cyber Orange™ gel stain.

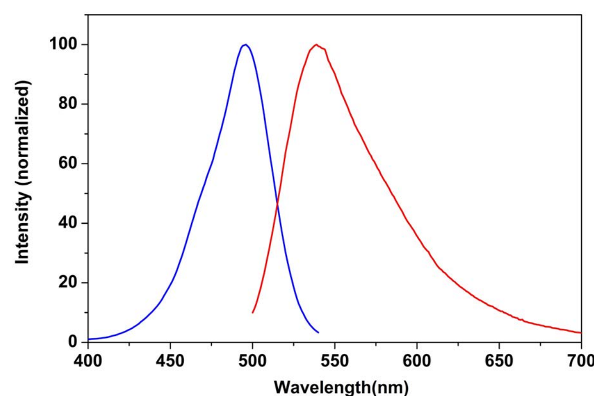


Figure 4.3. The excitation and emission spectra of Cyber Orange™ in the presence of calf thymus DNA.

Upon binding to nucleic acids, Cyber Orange™ Nucleic Acid Gel Stain exhibits a >1000-fold fluorescence enhancement. By comparison, ethidium bromide exhibits <30-fold fluorescence enhancement upon binding nucleic acids. Because of its superior fluorescence characteristics, Cyber Orange™ stain is 10-fold more sensitive than ethidium bromide for detecting DNA and RNA in gels using a 300 nm UV transilluminator and black-and-white photography (Figure 4.4). As little as 25 pg dsDNA or 1 ng RNA per band was detected using a 300 nm UV transilluminator or a blue-light transilluminator. The sensitivity levels are even higher than those of silver staining. Staining gels with Cyber Orange™ stain after electrophoresis followed by gel photography provides the optimal sensitivity.

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Cyber Orange™ gel stain penetrates agarose gels faster and stains thick and high percentage gels better than other post-electrophoresis stains. Cyber Orange™ gel stain is a universal nucleic acid gel stain that provides high sensitivity for dsDNA, ssDNA and RNA detection in many gel types, including high-percentage agarose, glyoxal/agarose, formaldehyde/agarose, native polyacrylamide– and urea–polyacrylamide gels. No wash step is required in order to achieve maximal sensitivities. As a result of the low intrinsic fluorescence of the unbound dye, gel staining with Cyber Orange™ dye shows extremely low background fluorescence and does not require a destaining step. After incubating the gel in Cyber Orange™ staining solution for 10–40 minutes, the orange fluorescent DNA or RNA bands are ready to be photographed.

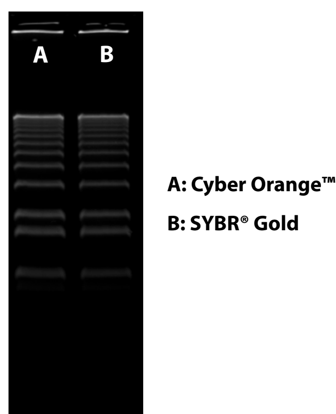

Figure 4.4. DNA molecular weight ladders stained with Cyber Orange™ (Cat# 17595) and SYBR® Gold Nucleic Acid Gel Stain.

The presence of unbound Cyber Orange™ dye in gels at standard staining concentrations does not interfere with restriction endonuclease or ligase activity or with subsequent PCR reactions. Cyber Orange™ nucleic acid staining is compatible with both Northern and Southern blotting—the stain transfers with the DNA or RNA to the blot and is washed off during incubation in the prehybridization mix. Cyber Orange™ stain is also easily removed from dsDNA by simple ethanol precipitation, leaving templates ready for subsequent manipulation or analysis. Our Gelite™ Orange Nucleic Acid Gel Staining Kit (Cat# 17594) includes our Cyber Orange™ nucleic acid stain with an optimized and robust protocol. It provides a convenient solution for staining nucleic acid samples in gels.

4.3 Other Dyes for Staining DNA in Gel Electrophoresis

The extraordinary stability of DiTO™–nucleic acid complexes allows the dye–DNA association to remain stable, even during electrophoresis. The nucleic acid samples can be stained with nM DiTO™-1 prior to electrophoresis, thereby reducing the hazards inherent in handling large volumes of ethidium bromide staining solutions. In contrast, the DNA binding of TO, the parent compound of DiTO™-1 is rapidly reversible, limiting the dye's sensitivity and rendering its nucleic acid complex unstable to electrophoresis. DNA samples can be prestained with nanomolar DiTO™ concentrations before electrophoresis. The fluorescence intensities of DiTO™-1–DNA complexes are directly proportional to the amount of DNA in a band. DiTO™-1 dye staining has little effect on the electrophoretic mobility of DNA fragment. The translocation of DiTO™-1 dye to unlabeled DNA is minimal with DiTO™-1–DNA complexes. This property is valuable for multiplexed electrophoretic separations. The binding of the DiTO™-1 dye to DNA initially results in inhomogeneous binding that yields double bands in DNA gel electrophoresis. The double bands can be avoided by incubating complexes long enough to allow binding to come to equilibrium or by heating samples to 50°C for at least two hours.

DiTO™-1 dye has been used to label DNA prior to electrophoresis in order to detect cystic fibrosis mutant alleles with a laser-excited fluorescence gel scanner, as well as to detect DNA amplification products on agarose gels with standard UV transillumination. DiTO™-1 dye has also been used to label nine DNA fragments of the dystrophin gene simultaneously generated using the polymerase chain reaction. The resolution obtained by gel electrophoresis of these labeled fragments is compared favorably to that observed using fluorophore-labeled primers. DiTO™-3 has been similarly used to analyze DNA with a xenon lamp–based luminescence analyzer.

Table 4.1 Nucleic Acid Gel Staining Probes and Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
17590	Cyber Green™ Nucleic Acid Gel Stain *10,000X DMSO Solution*	1 mL	497	520
17595	Cyber Orange™ Nucleic Acid Gel Stain *10,000X DMSO Solution*	1 mL	495	540
17575	DiTO™-1 [equivalent to TOTO®-1] *1 mM DMSO Solution*	0.2 mL	514	535
17576	DiTO™-3 [equivalent to TOTO®-3] *1 mM DMSO Solution*	0.2 mL	642	660
17580	DiYO™-1 [equivalent to YOYO®-1] *1 mM DMSO Solution*	0.2 mL	491	509
17581	DiYO™-3 [equivalent to YOTO®-3] *1 mM DMSO Solution*	0.2 mL	612	631
17589	Gelite™ Green Nucleic Acid Gel Staining Kit	1 Kit	497	520
17594	Gelite™ Orange Nucleic Acid Gel Staining Kit	1 Kit	495	540
17571	TWO-PRO™ 1 [equivalent to TO-PRO® 1] *1 mM DMSO Solution*	0.2 mL	491	509
17572	TWO-PRO™ 3 [equivalent to TO-PRO® 3] *1 mM DMSO Solution*	0.2 mL	612	631

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Tel: 800-990-8053 • Fax: 408-733-1304
sales@aatbio.com • info@aatbio.com

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Austria:
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Email: info@assaymatrix.com
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Canada:
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China:
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Croatia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Czech Republic:
Scintila, s.r.o.
Email: rejtharkova@scintila.cz
Website: http://www.scintila.cz

Denmark:
Nordic BioSite ApS
Email: info@nordicbiosite.dk
Website: http://www.nordicbiosite.dk

Estonia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Finland:
Nordic BioSite OY
Email: info@biosite.fi
Website: http://www.biosite.fi

France:
EUROMEDEX
Email: research@euromedex.com
Website: http://www.euromedex.com

Interchim
Email: interchim@interchim.com
Website: http://www.interchim.com

Germany:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Hong Kong:
Mai Bio Co., Ltd
Email: info@maibio.com
Website: http://www.maibio.com

Hungary:
IZINTA Trading Co., Ltd.
Email: baloghk@izinta.hu
Website: http://www.izinta.hu

Iceland:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

India:
Biochem Life Sciences
Email: info@bcls.in
Website: http://www.bcls.in

GenxBio Health Sciences Pvt. Ltd,
Email: sales@genxbio.com
Email: genxbio@gmail.com
Website: http://www.genxbio.com

Ireland:
Stratech Scientific Ltd.
Email: info@stratech.co.uk
Website: http://www.stratech.co.uk

Israel:
ADVANSYS Technologies for Life Ltd.
Email: info@advansys.co.il
Website: http://www.advansys.co.il

Italy:
Space Import Export S.r.l.
Email: info@spacesrl.com
Website: http://www.spacesrl.com

Valter Occhiena S.r.l.
Email: vo@valterocchiena.com
Website: http://www.valterocchiena.com

Japan:
Cosmo Bio Co., Ltd.
Email: mail@cosmobio.co.jp
Website: http://www.cosmobio.co.jp

Nacalai Tesque, Inc.
Email: info@nacalaiusa.com
Website: http://www.nacalai.com

Wako Pure Chemical Industries, Ltd.
Email: labchem-tec@wako-chem.co.jp
Website: http://www.wako-chem.co.jp

Korea:
Cheong Myung Science Corporation
Email: cms@cmscorp.co.kr
Website: http://www.cmscorp.co.kr

Kimnfriends Corporation
Email: kimnfriends@hanmail.net
Website: http://www.kimnfriends.co.kr

Latvia and Lithuania:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Netherlands:
ITK Diagnostics BV
Email: info@itk.nl
Website: http://www.itk.nl

Norway:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Poland and Slovenia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Romania:
SC VitroBioChem SRL
Email: office@vitro.ro
Website: http://www.vitro.ro

Singapore and Other South Asian Countries:
BST Scientific Pte Ltd.
Email: info@bstsci.com
Website: http://www.bstsci.com

Slovakia:
Scintila, s.r.o.
Email: rejtharkova@scintila.cz
Website: http://www.scintila.cz

South American Countries and Regions:
Impex Comércio Internacional Ltda.
Email: impexcom@terra.com.br
Website: http://www.impexbrasil.com.br

Spain and Portugal:
Deltaclon S. L
Email: info@deltaclon.com
Website: http://www.deltaclon.com

Sweden:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Switzerland:
LuBioScience GmbH
Email: info@lubio.ch
Website: http://www.lubio.ch

Taiwan:
Cold Spring Biotech Corp.
Email: csbiotech@csbiotech.com.tw
Website: http://www.csbiotech.com.tw

Rainbow Biotechnology Co., LTD.
Email: rainbow@rainbowbiotech.com.tw
Website: http://www.rainbowbiotech.com.tw

Turkey:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

United Kingdom:
Stratech Scientific Ltd.
Email: info@stratech.co.uk
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AAT Bioquest®, Inc.
520 Mercury Drive
Sunnyvale, CA 94085, USA
Tel.: 800-990-8053/408-733-1055
Fax: 408-733-1304
Email: sales@aatbio.com

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