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WHITE PAPER

ImmunoEconomics:

Financial Considerations for In-House Manual Cell Isolation Versus Cryopreserved Cell Products

Introduction

Researchers must carefully plan their experiments to yield the maximum amount of data while keeping to a budget. This can be especially challenging for immune cell assays that require a specific cell subset, such as cytotoxic CD8+ T-cells, because manual cell isolations from whole blood can accrue significant reagent and labor costs. In this cost analysis, we explore a standard CD8+ T-cell culture initiation protocol to determine the potential labor and material savings from using cryopreserved cell products as compared to using whole blood as a starting material.

Model Procedure & Assumptions

There are many different procedures to manually isolate cells from whole blood. In this analysis, we describe two workflows to generate cultures derived from negatively selected CD8+ cytotoxic T cells. The traditional **Manual Isolation Workflow** starts with 210 mL of whole blood. We assume that this starting material contains $40x10^6$ CD8+ T-cells. For the sake of simplicity, a single RBC lysis step is performed, as well as a single negative isolation step. Alternatively, the **Cryopreserved Cell Inventory Workflow** begins with two cryopreserved vials, each with 5M negatively selected CD8+ T-cells.

For both workflows:

- An activation step is required prior to cell culture initiation.
- We assume a starting cell density of $5x10^5$ cells/mL in 10 mL within each cell culture flask.[1]
- We assume a labor and overhead rate of $130 per hour.
- Consumable costs such as latex gloves, cleaning solutions and pipette tips are omitted.

Step	Manual Isolation Workflow	Cryopreserved Cell Inventory Workflow
1	Dilute whole blood with PBS 1:1	Thaw vial
2	Add 15 mL density gradient to tubes	Add media/cells to 50 mL tube
3	Centrifuge	Centrifuge
4	Isolate PBMC layers/consolidate	Wash/centrifuge cells
5	Wash/Centrifuge cells	Resuspend in media
6	Add RBC lysis buffer	QC Purity: Antibody Staining (optional)
7	Centrifuge	QC Purity: Centrifuge (optional)
8	Wash/Centrifuge cells	QC Purity: Flow Analysis (optional)
9	Resuspend in media	Enumerate cells
10	Enumerate cells	Add media to desired cell density
11	Centrifuge	Add activators
12	Resuspend in appropriate volume	Initiate culture
13	Add isolation reagent	
14	Incubate	
15	Discard deselected cells	
16	Wash/Centrifuge cells	
17	Resuspend in media	
18	Enumerate cells	
19	QC Purity: Antibody Staining	
20	QC Purity: Centrifuge	
21	QC Purity: Flow Analysis	
22	Add media to desired cell density	
23	Bank remaining cells	
24	Add activators to cultures	
25	Initiate culture	

Product brand names are blinded for the purposes of this evaluation. Prices were obtained from vendor websites in December 2020.

Material	Unit Size	Price (USD)
Phosphate-buffered Saline	500 mL	$22.48
Serum-free T cell medium	1000 mL	$166.00
Serum-free supplement	50 mL	$170.00
Density gradient medium	600 mL	$190.14
50 mL conical tubes	500 tubes	$415.00
1X RBC lysis buffer	200 mL	$55.00
Activation kit	1 kit	$980.00
Negative isolation kit	1 kit	$794.00
IL-2 growth factor	10 µg	$268.00
Automated cell counter cassette	100 cassettes	$285.00
Cell culture flasks	100 flasks	$390.00
Cryopreservation media	100 mL	$350.00
1.2 mL Cryovials	1000 vials	$721.59
Anti-human CD3 Antibody	25 tests	$20.00
Anti-human CD8 Antibody	25 tests	$120.00

Just as there are many different isolation procedures, there are also many cell culture media formulations. For the sake of simplicity, we assume a serum-free T-cell culture medium completed with 5% of serum-replacement solution and 300IU/mL of IL-2 cytokine. Given the prices above, this equates to approximately $0.41 per mL of completed media.

Cost Analysis

As you can see in the table, the **Manual Isolation Workflow** is much more involved than the **Cryopreserved Cell Inventory Workflow**. Transferring the whole blood to individual conical tubes, diluting with PBS, adding density gradient media and centrifuging takes an hour of labor. The separation of PBMCs without contamination from RBCs also requires a skilled operator, as RBC contamination can have deleterious effects on cell culture. The inclusion of a negative isolation kit is also a major cost contributor to the **Manual Isolation Workflow**.

Step	Manual Isolation Workflow	Touch Points	Materials Used	Labor Time (m)	Costs
1	Dilute whole blood with PBS 1:1	X	14 x 50 mL tubes, 200 mL PBS	20	$ 20.61
2	Add 15 mL density gradient to tubes	X	210 mL Ficoll	10	$ 66.55
3	Centrifuge		Centrifuge	30	$ -
4	Isolate PBMC layers/consolidate	X	1 x 50 mL tube	15	$ 0.83
5	Wash/Centrifuge cells	X	50 mL PBS, Centrifuge	15	$ 2.25
6	Add RBC lysis buffer	X	50 mL RBC	5	$ 13.75
7	Centrifuge		Centrifuge	15	$ -
8	Wash/Centrifuge cells	X	50 mL PBS, Centrifuge	15	$ 3.17
9	Resuspend in media	X	50 mL complete media	5	$ 20.59
10	Enumerate cells	X	1 kit	5	$ 2.85
11	Centrifuge		Centrifuge	15	$ -
12	Resuspend in appropriate volume	X	~50 mL complete media	5	$ 20.59
13	Add isolation reagent	X	1 kit	5	$ 794.00
14	Incubate		-	10	$ -
15	Discard deselected cells	X	-	2	$ -
16	Wash/Centrifuge cells	X	50 mL PBS, Centrifuge	15	$ 2.85
17	Resuspend in media	X	50 mL complete media	5	$ 20.59
18	Enumerate cells	X	1 kit	5	$ 2.85
19	QC Purity: Antibody Staining	X	CD3+, CD8+ stains	10	$ 5.60
20	QC Purity: Centrifuge		Centrifuge	15	$ -
21	QC Purity: Flow Analysis		-	10	$ -
22	Add media to desired cell density	X	200 mL complete media, 2 culture flasks	5	$ 90.17
23	Bank remaining cells	X	10 mL CryoStor CS10, 10 Cryovials	30	$ 42.22
24	Add activators to cultures	X	1 kit	5	$ 980.00
25	Initiate culture		Incubator	5	$ -
			Totals	**4h 37m**	**$2,689.64**

Altogether, the **Manual Isolation Workflow** takes 4 hours and 37 minutes and incurs a total cost of $2,689.64.

The **Cryopreserved Cell Inventory Workflow**, by comparison, has considerably fewer touch points and a shorter duration to complete.

Step	Cryopreserved Cell Inventory Workflow	Touch Points	Materials Used	Labor Time (m)	Costs
1	Thaw vial		Waterbath	5	$ 20.61
2	Add media/cells to 50 mL tube	X	50 mL complete media	5	$ 66.55
3	Centrifuge	X	Centrifuge	5	$ -
4	Wash/Centrifuge cells	X	50 mL PBS, centrifuge	10	$ 0.83
5	Resuspend in media	X	50 mL complete media	5	$ 2.25
6	QC Purity: Antibody Staining	X	CD3+, CD8+ stains	10	$ 13.75
7	QC Purity: Centrifuge		Centrifuge	15	$ -
8	QC Purity: Flow Analysis		-	10	$ 3.17
9	Enumerate cells	X	1 kit	5	$ 20.59
10	Add media to desired cell density	X	200 mL complete media, 2 flasks	5	$ 2.85
11	Add activators	X	1 kit	5	$ -
12	Initiate culture		Incubator	5	$ 20.59
			Totals	**1h 20m**	**$1,333.12**

Only 1 hour and 20 minutes are required for culture initiation, at a total cost of $1,333.12 – a savings of $1,356.52 and 3 hours and 17 minutes. Many researchers will attempt to recover this cost difference by banking the remaining cells from the whole blood isolation. However, it is important to note that the comparatively high number of operator touch points (19 in total) increases the odds of microbial contamination within the cryovial. Subsequent use of the banked cells will also introduce an additional eight touch points, as the **Cryopreserved Cell Inventory Workflow** is needed to begin the culture after thawing. Using ready-to-ship CD8+ T-cells as a starting product also provides an additional level of confidence, as quality control (QC) release specifications guarantee the product's purity.

Key Considerations

It can be difficult to realize the cost advantages of scale in a single research laboratory isolating immune cells. If reagent costs cannot be spread out across a large cell bank, they can quickly deplete an annual budget. Labor time spent on cellular isolation can also detract from investigative research. This is a troubling opportunity cost, especially in therapeutic development, where time-to-market is a key consideration. Scheduling laboratory time has also become an issue since the COVID-19 pandemic, as capacity restrictions limit the number of employees allowed in a laboratory space. Under these conditions, it is best to optimize the available time for application-based work, rather than cellular preparation.

Economics aside, researchers must weigh the risk of potential contamination from additional touch points against the budgetary considerations for starting materials. The purchase of immune cell subsets serves as a meaningful strategy to reduce risk, save on material costs and recoup opportunity costs so researchers can focus on their science.

BioIVT's inventory of ready-to-ship cryopreserved immune cell subsets is well-characterized and updated weekly. Start saving time and money today by reviewing our services.

References

1. Ou, J., Si, Y., Tang, Y., Salzer, G. E., Lu, Y., Kim, S., Qin, H., Zhou, L., & Liu, X. (2019). Novel biomanufacturing platform for large-scale and high-quality human T cells production. Journal of biological engineering, 13, 34. [Link to Pubmed Article]

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