Immune Cell Isolation from Whole Blood and Leukopaks

High viability, high-purity PBMCs and lymphocytes for biomarker studies and research

Maximize Viability

Processing within 24 hrs of initial collection

Cells per aliquot: 5–10 million (WB) 25 or 100 million (leukopak)

Target cell viability*:
- PBMCs: ≥95%
- T, B, NK cells: ≥90%

Fresh delivery in certain geographies

*disease-state dependent

Flexibility & Customization

Compliance is assured through standard protocols or provided SOPs

Dedicated study coordinator manages logistics and requests

Convenience & Efficiency

One experienced partner from study participant recruitment to specimen delivery

Make the most of your lab space and personnel

PBMC and lymphocyte isolations from leukopaks & whole blood for RUO and clinical trials

Peripheral blood mononuclear cells (PBMCs) include lymphocytes (T cells, B cells, NK cells), monocytes, and neutrophils and are the workhorses of modern therapy development. PBMC phenotypes and activities represent valuable biomarker sources for disease progression, drug efficacy, and safety. Specific subtypes are commonly selected and engineered for cell & gene therapies. For their value to be realized in R&D, PBMC viability is paramount. Viability begins to decrease as soon as the cells are removed from the body and is heterogenous in relation to cell type; processing delays complicate translational and clinical results and should be avoided.[1,2]

Choosing a single, capable partner for patient recruitment, biospecimen collection, and immune cell isolation can improve PBMC quality by reducing the chain of custody and time samples spend out of the body and under regulated temperatures. Think of Sanguine as an extension of your lab's capabilities. You can rely on our validated SOPs or provide your own. Save time and lab space by choosing an experienced and dedicated preanalytical services provider. We will customize our processes to meet and exceed your expectations.

Isolating T cells, B cells, and NK cells from peripheral blood

We use magnetic isolation kits to extract lymphocytes from leukopaks and whole blood. Negative selection is used by default, whereby cell types other than the specified cell type are targeted and magnetically depleted, leaving the cells of interest untouched in solution. However, we can perform a positive selection upon request.

Get the immune cells you need for your research

Collection	PBMCs	T cells	B cells	NK cells
Leukopaks	Density gradient isolation	Negative selection	Negative selection	Negative selection
Whole Blood				N/A
Whole Blood for Clinical Trials		N/A	N/A	N/A

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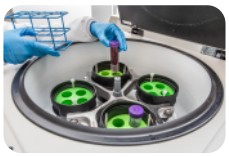
Sanguine

PBMC isolation and aliquoting at Sanguine's San Diego, CA laboratory

LEUKOPAKS

WHOLE BLOOD

Dilute specimen 2:1 with wash buffer and distribute to 50 mL conical tubes containing density gradient medium (>2:1 specimen : medium)



Centrifuge the settled specimen plus density gradient medium at 400 x g for 30 min, stopping with the brake off.

Remove and discard the plasma layer, and transfer the PBMC layer to a fresh 50 mL conical tube.

Top up the tube with wash buffer, mix, and centrifuge at 300 x g for 10 minutes with the low brake on. Remove the supernatant and repeat.

If RBCs are visible, incubate with lysis buffer (9:1) for 10 minutes, wash and centrifuge, and remove the supernatant.

Measure the cell count and viability with an automated cell counter. Determine the volume of CryoStor CS10 freezing media to give max # cells.

Wash PBMCs, centrifuge, and discard the supernatant. Add an appropriate amount of freezing media to the PBMCs prior to aliquoting.

Aliquot PBMCs plus freezing media into 2 mL cryovials. Freeze the samples in a Mr. Frosty or control rate freezing device at -80°C for 4 hrs.

Case Study: PBMC processing for a clinical trial site in San Diego, CA

A large biopharmaceutical company partnered with Sanguine to process PBMCs from whole blood collected from clinical trial participants in the greater San Diego metro area. Sanguine:

- Couriered the whole blood specimens from the clinical site to the lab
- Isolated PBMCs within four hours of collection
- Cryopreserved the cell isolates
- Shipped them on dry ice to the client.

Upon two weeks of cryostorage, the client reported cell count viability data of the thawed specimens, presented in **Table 1.** The Sanguine lab outperformed the client's acceptance viability criteria of ≥90% upon thawing and ≥85% after resting overnight.

"Reducing PBMC processing delays may be an important consideration when designing sample collection protocols in clinical trials to minimize the impact of preanalytical factors on downstream assays."

-Ping-Cheng Yi and colleagues, *Journal of Immunological Methods*[1]

Table 1. Cell counts and viabilities for Sanguine lab processing of clinical PBMCs

Sample ID	Upon Thawing		After Resting Overnight
	Total Viable Cells (x 10^6)	% Viability	% Viability
Technician A-1	5.51	99	87
Technician A-2	5.20	100	86
Technician B-1	5.36	100	94
Technician B-2	5.30	99	93
Technician C-1	5.39	98	87
Technician C-2	5.61	99	89
Technician D-1	5.72	100	93
Technician D-2	5.45	98	95

[1]Yi P-C, et al. (2023) Impact of delayed PBMC processing on functional and genomic assays. *J Immunol Methods*. 519: 113514.
[2]Ellervik C & Vaught J. (2015) Preanalytical variables affecting the integrity of human biospecimens in biobanking. *Clin Chem*. 61: 914–934.