Product Description

During routine plateletpheresis, platelets are separated from other blood components by centrifugation. Leukoreduction system (LRS) chambers play a direct role in the cell reduction process used to collect the platelets. Upon completion of the donation process, red blood cells, white blood cells, and plasma are returned to the donor. However, a small volume of granulocyte-reduced, concentrated mononuclear cells remains inside the LRS chamber (Figure 1A and Table 1). These chambers are typically discarded following the completion of the platelet donation process, but if they are sterilely separated from the collection kit, the mononuclear cells contained within can be harvested for use in a whole host of research applications1-4.

Work from our group shows an average of 1.8x10^9 total nucleated cells (TNC, Figure 1B and Table 1) are obtained from a single LRS chamber, with an average of 96.3% of those cells being lymphocytes and monocytes (Mononuclear Cells, Figure 1A and Table 1). CD3+ T cells make up between 30% and 65% of the total LRS chamber contents, depending on the individual (Table 1). The distribution of common mononuclear subpopulations is shown in Figure 1C and listed in Table 1.

Figure 1: Makeup of LRS chambers from platelet-only donors collected at the Oklahoma Blood Institute. A. The percentage of mononuclear cells (lymphocytes and monocytes) and granulocytes (neutrophils, eosinophils, basophils) in LRS Chambers at time of harvest. Percentages obtained from Sysmex Hematology Analyzer. B. Absolute cell numbers counted by Sysmex Hematology Analyzer (for TNC [Total Nucleated Cells], Lymphocytes, and Monocytes) or by flow cytometry (T cells). C. Absolute number of mononuclear cell subpopulations in LRS chambers, calculated from flow cytometry percentage multiplied by chamber TNC. See Table 1 for mean and range for each population.

Table 1: Percentages and absolute cell numbers of LRS chamber populations. Total Nucleated Cells, Granulocytes (neutrophils, eosinophils, basophils), Mononuclear Cells (Lymphocytes, Monocytes) were measured by Sysmex hematology analyzer. T Cells (CD3+, CD4+, CD8+), B Cells, CD14+ Monocytes, and NK Cells were measured by flow cytometry and absolute cell numbers were calculated by multiplying the percentage by the total nucleated cell count. The mean and range for percentage and absolute number are reported for all populations.

Storage
Cells harvested from LRS chambers in media or plasma may be stored at 4°C for 24-96 hours and maintain up to 90% total viability (Figure 2A). Chambers may be stored, unharvested for up to 48h at 4°C with >95% total viability expected upon harvest or for up to 96h at 4°C with >90% total viability expected upon harvest (Figure 2B).

Recommended LRS Chamber Harvest Protocol

Materials
1. Ring stand with clamp
2. 50 mL conical tube
3. Conical tube rack
4. 70% ethanol or isopropanol
5. Scissors
6. Blunt-end 18G needle
7. 20 mL syringe
8. 40 mL of Harvesting Media: PBS or culture media supplemented with BSA, HSA, or serum
9. Hemocytometer or automated cell counter

Protocol
Cells inside LRS chambers are sterile. To maintain sterility, perform all steps inside a biological safety cabinet, practice sterile technique, and use only sterile supplies and media.

1. Mount LRS chamber on a ring stand with clamp, wide side down (Figure 3). Position tubing over a closed 50 mL conical tube sitting inside a tube rack.
2. Spray LRS chamber with alcohol and allow to dry.
3. Open 50 mL tube and snip the bottom tubing but leave ~½ inch of slack (Figure 3). Repeat for the top tubing, which will break the vacuum and the chamber will start dripping into the open tube.
4. For best results, allow the contents of the chamber (~10 mL) to drip by gravity into the tube (takes ~10 min)*.
   *If this is going very slowly or has stopped, take the syringe and blunt-end needle and inject air into the top tubing to resume drip.
5. Fill syringe up with 20 mL of Harvesting Media and inject into the top of the chamber**. Allow all media to drip into the tube.
   **Move the tubing in a circle while slowly injecting the Harvesting Media to help clear out blood from the sides of the chamber.
6. Repeat with another 20 mL of Harvesting Media for a total volume of ~50 mL of LRS eluate + Harvesting Media.
7. Fill syringe with air and push air through the chamber to expel any remaining volume. The chamber should be cleared of most blood and cells (Figure 3), although trace amounts may remain.
8. Cap tube and gently mix by inverting.
9. Remove a sample for counting and proceed to downstream research applications.

Warning
This product is composed of human-derived materials. Always wear appropriate personal protective equipment when handling this product and treat it as potentially infectious, using Universal Precautions, regardless of the results of infectious disease testing.

Limitations and Publications
This product is for research use only and not for use in humans, for further manufacture, or resale. Nothing produced directly from this product may be sold. When publishing scientific results obtained using this product, acknowledge supplier as Bio-Sharing.org.