Lyophilized Human PBMCs, Veri-Cells™, Superior Controls for Flow Cytometry Applications

Divekar AA, Rogers MT, Lee MJ, Gordon M, Soper DM, Yang XF, Monell C, Ransom J, Lay G
BioLegend, 9727 Pacific Heights Blvd, San Diego, CA 92121

Abstract

Reference laboratories, clinical research organizations and multi-center clinical trials need stabilized cells as controls to validate lot-to-lot consistency and for daily machine set up. We have developed a lyophilized peripheral blood mononuclear cell (Lyo-PBMC) preparation that can be successfully used for detecting a wide range of surface markers (on resting and activated PBMC), including CD3, CD4, CD8, CD16, CD19, CD20, CD21, CD22, CD25, CD27, CD56, CD69, CD154, CD360 and IL-21R. Additionally, Lyo-PBMCs can be used for detecting intracellular cytokines, such as IL-2, IFN-γ and TNF-α, and transcription factors, such as Foxp3. Upon reconstitution, Lyo-PBMCs maintain forward/side scatter profiles (of lymphocytes and monocytes) similar to freshly isolated PBMC, and expression of the above mentioned markers is stable. Ongoing studies are investigating the long term stability (> 6 months) of Lyo-PBMC preparations.

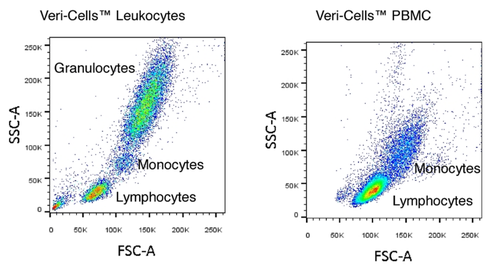
Introduction

Flow cytometry assays involve multiple reagents, including fluorescent antibodies and buffers. Patient samples may or may not express certain markers; therefore, it is important to confirm that the multi-color cocktails prepared each day can detect all markers of interest. Reference and clinical laboratories also need to ensure that all reagents give similar performance each day. To this end, a control cell population is run along with the samples. Currently, several control cell products are available on the market, but most have limitations relative to stability. Closed vial shelf life is generally only a few months, and open vial stability can be only a few days. Our new product, Veri-Cells™ are expected to have stable performance for most, if not all, claimed applications for at least a year as a closed vial and five days post reconstitution. Here we discuss the lyophilized cell products, Veri-Cells™ PBMC, Veri-Cells™ Leukocytes and Veri-Cells™ Activated PBMC. The scatter profile of these is similar to that of freshly prepared PBMC or lysed whole blood. Surface staining has shown stable expression of several common markers, such as CD3, CD4, CD8, CD16, CD19, CD20, CD21 and CD56. Additionally, we have been able to detect intracellular cytokines such as IL-2, IFN-γ, and TNF-α and transcription factors Foxp3 and Helios. The background fluorescence of the cells is similar to normal human samples, which is a significant improvement over other stabilized controls currently available. Customized control products with assay values can also be designed, and large lot sizes may be ordered for specific customer needs.

Methods

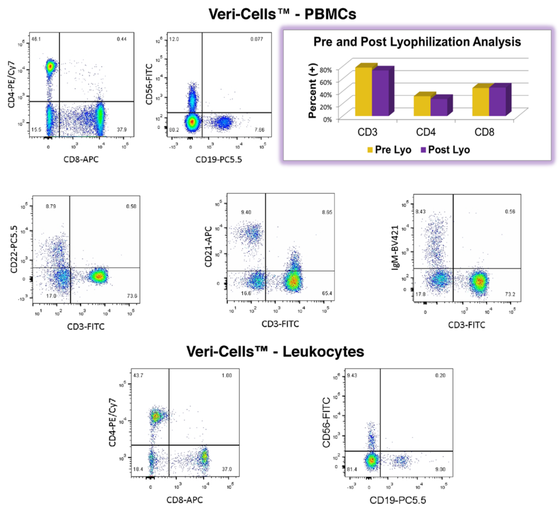
Lyophilized control cells were reconstituted with the Reconstitution buffer (included). The cells were stained at recommended antibody concentrations and washed twice with an isotonic wash buffer. Samples were analyzed on either BD™ or Beckman Coulter™ flow cytometers.

Figure 1. Lymphocyte, monocyte and granulocyte scatter profile is preserved post lyophilization



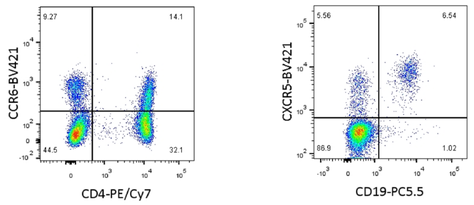
Veri-Cells™ – Leukocytes or PBMC were reconstituted with Reconstitution buffer and acquired on a flow cytometer such BD FACSCanto™ II. Distinct lymphocyte, monocyte and granulocyte populations could be identified.

Figure 2. Surface marker expression of the Veri-Cells™ PBMC and Leukocytes is stable post lyophilization



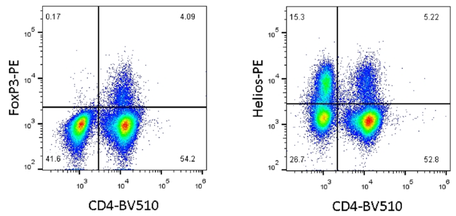
Veri-Cells™ – Leukocytes or PBMC were reconstituted with Reconstitution buffer and surface stained with CD4 PE/Cy7, CD8 APC, CD19 PerCP/Cy5.5 and CD56 FITC. Dot plots are gated on the lymphocyte population.

Figure 3. Chemokine receptors can be detected post lyophilization on Veri-Cells™ PBMCs



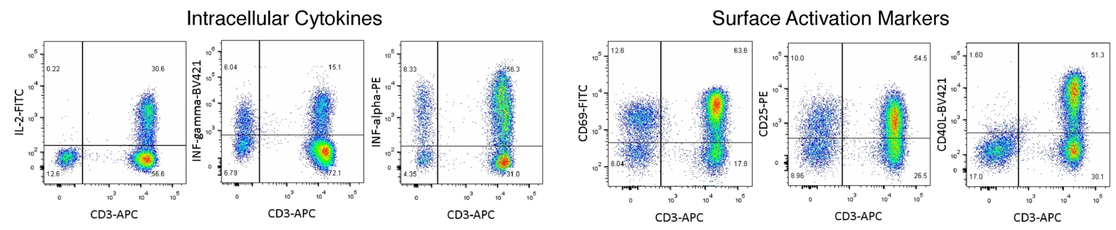
Veri-Cells™ –PBMC were reconstituted with Reconstitution buffer and were surface stained with CD4 PE/Cy7, CD19 PerCP/Cy5.5, CXCR5 PE/Dazzle™ 594 and CCR6 Brilliant Violet 421™. Dot plots are gated on the lymphocyte population.

Figure 4. Transcription factors Foxp3 and Helios could be detected in Veri-Cells™ PBMC



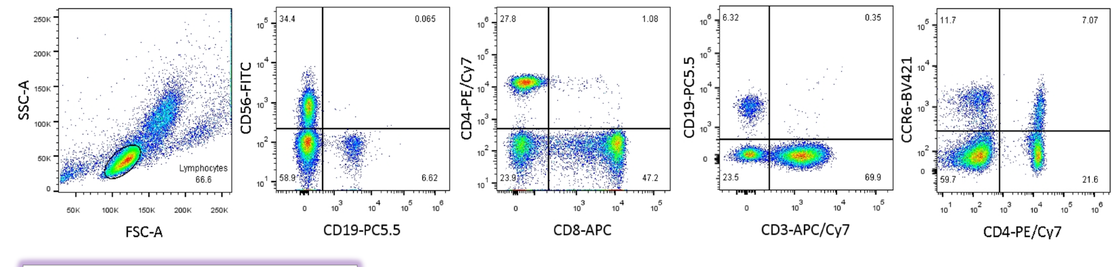
Veri-Cells™ –PBMC were reconstituted with provided Reconstitution buffer and surface stained with CD4 Brilliant Violet 510™, then fixed and permeabilized with True-Nuclear™ Transcription Factor buffer set followed by intra-nuclear staining with Foxp3 PE or Helios PE. Staining pattern for Helios and Foxp3 is very similar to that observed with freshly isolated PBMC. Dot plots are gated on the lymphocyte population.

Figure 5. Intracellular cytokines and surface activation marker expression in Veri-Cells™ Activated PBMC is maintained post lyophilization



Veri-Cells™ – Activated PBMC were reconstituted with Reconstitution buffer and surface stained with CD3, IL-2, IFN-γ, and TNF-α or with CD3, CD69, CD25 and CD40L(CD154). Dot plots are gated on the lymphocyte population.

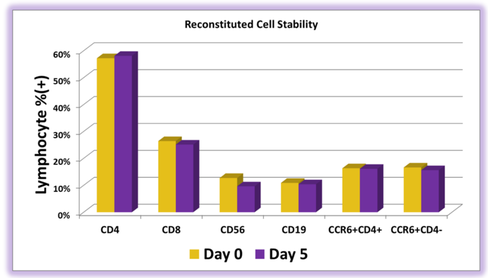
Figure 6. Excellent long term stability (closed vial) observed with Veri-Cells™ PBMC



Veri-Cells™ –PBMC (10 month time point) were reconstituted with Reconstitution buffer and surface stained with CD4 PE/Cy7, CD8 APC, CD19 PerCP/Cy5.5, CD56 FITC and CCR6 Brilliant Violet 421™. Dot plots are gated on the lymphocyte population (top panel).

Frequencies of CD3, CD4, CD8 positive T cells and B cells were almost identical between Veri-Cells™ PBMC tested immediately post lyophilization and Veri-Cells™ PBMC stored at 4°C for 10 months (left chart).

Figure 7. Reconstituted Veri-Cells™ PBMC can be used for five days post reconstitution without significant loss of signal



Veri-Cells™ –PBMC were reconstituted with Reconstitution Buffer and surface stained with CD4 PE/Cy7, CD8 APC, CD19 PerCP/Cy5.5, CD56 FITC and CCR6 Brilliant Violet 421™ on day 0 and day 5 post-reconstitution. Dot plots are gated on the lymphocyte population.

Conclusions

1. The scatter patterns of normal, human Leukocytes and PBMCs are preserved with our technology, allowing easy identification of lymphocytes, monocytes and granulocytes post lyophilization.
2. Background fluorescence and most pattern and intensity staining performance metrics are equivalent to freshly prepared leukocytes.
3. The frequencies of CD3, CD4 and CD8 positive cells post lyophilization are similar to pre lyophilization testing, indicating excellent epitope preservation.
4. Veri-Cells™ PBMC can be used to monitor reagent performance for most common surface molecules but also for activation markers such as CD69, CD25 and CD154
5. Robust expression of transcription factors such as Foxp3 and Helios was detected and mimicked that which is observed in fresh cells
6. Remarkable stability in both closed vial and reconstituted conditions offers flexibility, reduced waste and consistent reliability over long term experiments and trials.
7. A wide variety of custom products, such as activated/resting cells or specifically depleted populations can also be tailored to the user's needs by our Custom Solutions Team.

04-0061-00