Veri-Cells™ Leukocytes and CD4 Low PBMC, lyophilized human blood cells are reliable controls for flow cytometric assays

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Abstract

Control cell populations offer the ability to monitor assay performance and variability for longitudinal studies. Veri-Cells™ Leukocytes, a lyophilized cell preparation, is an excellent human immunophenotyping control, as it includes all leukocyte (lymphocytes, monocytes and granulocytes) subsets. Our Veri-Cells™ PBMC and CD4 Low PBMC are available as clinically useful controls, allowing monitoring of normal and low levels of $CD4^+$ cells. These cell preparations can be used to assay most CD markers and chemokine receptors such as CXCR5, CCR6, CCR4 and CCR7. Our Custom Solutions Team offers the option to tailor control cells to their specific requirements, ranging from pre-lyophilization staining with live/dead dyes, cell activation and selective depletion/enrichment. Lyophilized cell lines provide the convenience of on-demand testing without the need for expensive tissue culture equipment or incubation/contamination delays. Combinations of cell lines or sorted isolates can be added to leukocytes as analogs for abnormal or rare event staining controls. Single or multi-test custom lots can be manufactured at almost any size for use in long term or multisite clinical trials.

Introduction

Flow cytometry assays involve multiple reagents including fluorescent antibodies and buffers. Donors may or may not express certain markers; therefore, reference and clinical laboratories need to confirm that the multi-color cocktails prepared each day can detect all markers of interest. To this end, a control cell is run along with the samples. Currently, several control cell products are available in the market, but most have limited shelf life.

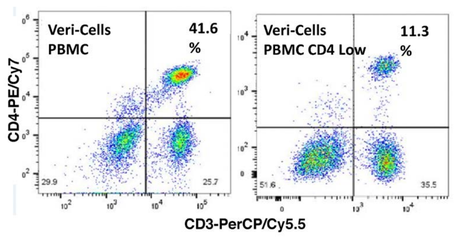
Veri-Cells™ are a line of lyophilized cell preparations, with stable performance for two years as a closed vial and five days post reconstitution. The scatter profile of our products is similar to that of freshly prepared cells. Using our LEGENDScreen™ Human Cell screening kit (Cat. No. 700001) to stain Veri-Cells™ PBMC, we have shown stable expression of >150 cell surface markers, including CD3, CD4, CD8, CD16, CD19, CD20, CD21, CD45 and CD56. Here we discuss Veri-Cells™ Leukocytes and Veri-Cells™ CD4 Low PBMC, which can be used for monoclonal antibody verification, instrument and operator validation. Veri-Cells™ CD4 Low PBMC can be paired with the Veri-Cells™ PBMC product as controls for clinically relevant levels of $CD4^+$ T cells.

Veri-Cells™ Leukocytes allow users to investigate expression of T, B and NK cell markers as well as monocyte and granulocyte markers. These cells can also be used to study intracellular molecules such as Granzyme B, Perforin, Foxp3, Helios, and T-bet.

Materials and Methods

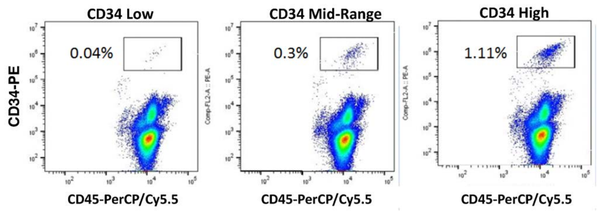
Veri-Cells™ PBMCs (Cat. No. 425001), Veri-Cells CD4 Low PBMC (Cat. No. 425601) and Veri-Cells™ Leukocytes were reconstituted with Veri-Cells Reconstitution buffer included in the kit. The cells were stained at recommended antibody dosages and washed twice with an isotonic wash buffer. Samples were acquired on either BD™ or Beckman Coulter™ flow cytometers and analyzed using FlowJo.

Figure 1. Veri-Cells™ PBMC and CD4 Low PBMC



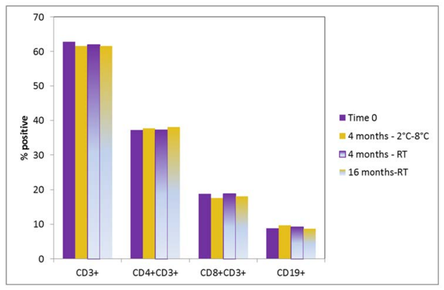
Veri-Cells™ products can be selectively enriched or depleted for cells of interest. The CD4 Low PBMCs have been modified to reduce CD4 positive T-Cells to within a specified range.

Figure 2. Control cells for enumeration of $CD34^+$ cells – Veri-Cells™ CD34 high, medium and low PBMC



KG1a cells were spiked into human PBMCs and lyophilized. The cells were reconstituted with Veri-Cells Reconstitution buffer and stained with CD34 PE and CD45 PerCP/Cy5.5.

Figure 3. Veri-Cells™ PBMC have excellent long term stability even at warm temperatures (20-25°C)



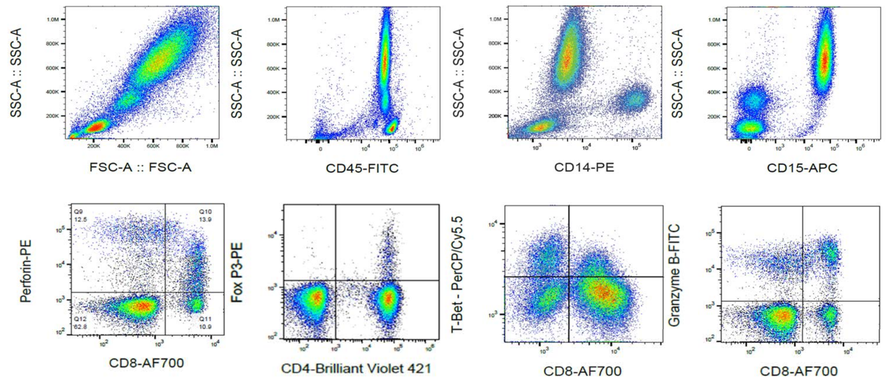
Veri-Cells™ PBMC were reconstituted with Veri-Cells™ Reconstitution buffer and then stained for T and B cell markers at 4 months and 16 months post lyophilization. Frequencies of the T cells, $CD4^+$ T cells, $CD8^+$ T cells and $CD19^+$ cells were stable over a period of 16 months.

Figure 4. >150 target antigens can be detected on Veri-Cells™ PBMC

Marker	Clone	Marker	Clone	Marker	Clone	Marker	Clone	Marker	Clone	Marker	Clone
CD1c	L161	CD29	TS2/16	CD54	HA58	CD97	VIM3b	CD165	SN2 (N6-D11)	CD314	1D11
CD1d	51.1	CD31	WM59	CD55	JS11	CD99	HCD99	CD172a	SE5A5	CD319	162.1
CD2	RPA-2.10	CD32	FUN-2	CD56	HCD56	CD100	A8	CD172b	B4B6	CD328	6-434
CD3	HIT3a	CD33	WM53	CD57	HCD57	CD101	BB27	CD172g	LSB2.20	CD335	9E2
CD4	RPA-T4	CD34	581	CD58	TS2/9	CD102	CBR-IC2/2	CD180	MHR73-11	CD337	P30-15
CD5	UCHT2	CD35	E11	CD59	p282 (H19)	CD107a	H4A3	CD182	5E8/CXCR2	CD352	NT-7
CD6	BL-CD6	CD36	5-271	CD61	VI-PL2	CD116	4H1	CD184	12G5	CD354	TREM-26
CD7	CD7-6B7	CD38	HIT2	CD62P	AK4	CD122	TU27	CD193	5E8	β2-microglobulin	2M2
CD8a	HIT8a	CD39	A1	CD63	H5C6	CD123	6H6	CD196	G034E3	CD272	MIH26
CD9	HI9a	CD40	HB14	CD64	10.1	CD124	G077F6	CD197	G043H7	C3aR	hC3aRZ8
CD11a	HI111	CD41	HIP8	CD69	FN50	CD126	UV4	CD200	OX-104	CLEC12A	50C1
CD11b	ICRF44	CD42b	HIP1	CD73	AD2	CD127	A019D5	CD200R	OX-108	CX3CR1	2A9-1
CD11b	CBRM1/5	CD43	CD43-10G7	CD74	LN2	CD132	TUGh4	CD226	11A8	FcRL6	2H3
CD11c	3.9	CD44	BJ18	CD79b	CB3-1	CD134	Ber-ACT35 (ACT35)	CD229	Hly-9.1.25	HLA-A,B,C	W6/32
CD13	WM15	CD45	HI30	CD81	5A6	4-1BB Ligand	5F4	CD244	C1.7	HLA-A2	BB7.2
CD14	M5E2	CD45RA	HI100	CD82	ASL-24	CD138	DL-101	CD268	11C1	HLA-DQ	HLADQ1
CD16	3G8	CD45RB	MEM-55	CD84	CD84.1.21	CD140b	18A2	HVEM	122	HLA-DR	L243
CD18	TS1/18	CD45RO	UCHL1	CD85	MKT5.1	CD148	A3	CD271	ME20.4	HLA-E	3D12
CD19	HIB19	CD46	TRA-2-10	CD85d	42D1	CD154	24-31	CD277	BT3.1	IgD	IA6-2
CD20	2H7	CD47	CC2C6	CD85	GHI/75	CD156c	SHM14	CD278	C398.4A	IgM	MHM-88
CD21	Bu32	CD48	BJ40	CD86	IT2.2	CD158a/h	HP-MA4	CD279	EH12.2H7	Integrin β7	FIB504
CD22	HIB22	CD49d	9F10	CD87	VIM5	CD158b	DX27	CD284	HTA125	Mac-2	Gal397
CD23	EBVCS-5	CD49e	NKI-SAM-1	CD88	S5/1	CD158e1	DX9	CD290	3C10C5	NKp80	5D12
CD24	ML5	CD49f	GoH3	CD89	A59	CD161	HP-3G10	CD298	LNH-94	Siglec-9	K8
CD26	BA5b	CD50	CBR-IC3/1	CD93	VIMD2	CD162	KPL-1	CD300e	UP-H2	TCR gamma/delta	B1
CD27	O323	CD52	HI186	CD94	DX22	CD163	GHI/61	CD300F	UP-D2	TCR Vβ13.2	H132
CD28	CD28.2	CD53	HI29	CD95	DX2	CD164	67D2	CD304	12C2	TCR Vβ23	αHUT7
TCR Vβ8	JR2 (JR.2)	TCR Vβ9	MKB1	TCR Vδ2	B6	Vγ9	B3	TCR Vα7.2	3C10	TCR α/β	IP26

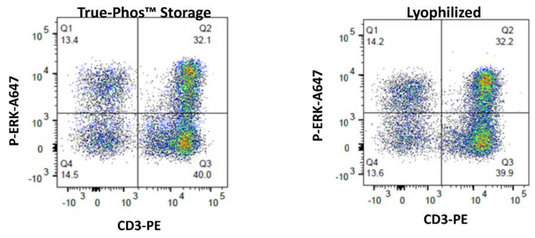
Veri-Cells™ PBMC were reconstituted with Veri-Cells™ Reconstitution buffer and then stained using the LEGENDScreen™ Human Cell Screening Kit (Cat. No. 700001). 168 target antigens were robustly identified on the Veri-Cells™ PBMC.

Figure 5. Veri-Cells™ Leukocytes maintain scatter, surface and intracellular markers post-lyophilization, similar to that of fresh leukocytes



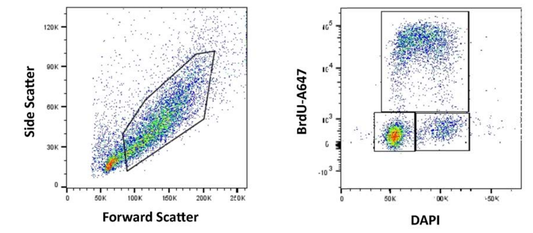
Whole blood was lysed and then lyophilized. The lyophilized cells were reconstituted with the Veri-Cells™ Reconstitution buffer, surface stained (CD4, CD8, CD14 and CD15) and then stained for intra-nuclear (Foxp3, T-bet) or intra cytoplasmic (Granzyme B and Perforin) markers using the True-Nuclear ™ Transcription Factor Buffer set (Cat. No. 424401) and Fixation/Permeabilization Buffer (Cat. No. 420801).

Figure 6. Veri-Cells™ Activated PBMCs, an innovative control for phospho signaling assays



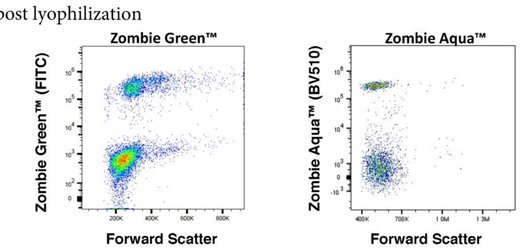
PBMCs were stimulated with Biolegend's Cell Activation Cocktail™ (Cat. No. 423301). Half of the cells were resuspended in True-Phos™ Permeabilization Buffer (Cat. No. 425401) and stored at -20°C. The other half was lyophilized. The lyophilized cells were reconstituted with Veri-Cells™ Reconstitution Buffer, permeabilized with True-Phos™ buffer and stained with CD3 PE and p-ERK.

Figure 7. BrdU loaded cells maintain label post lyophilization



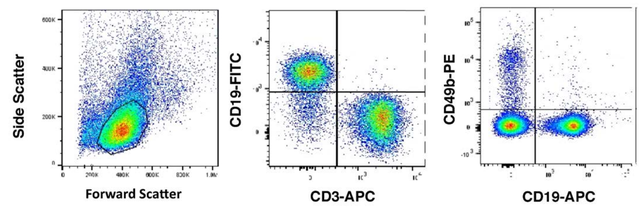
Human Th2 polarized PBMCs were pulsed with BrdU for 1 hour prior to processing and lyophilization. The cells were reconstituted with Veri-Cells™ Reconstitution Buffer, fixed and treated with DNAse for 1 hour at 37°C and then stained with BrdU-Alexa Fluor® 647 and DAPI.

Figure 8. Veri-Cells PBMC pre-stained with live/dead discriminator (Zombie™ dyes) maintain expression post lyophilization



Veri-Cells PBMCs were stained with Zombie Green™ (Cat. No. 423111) or Zombie Aqua™ (Cat. No. 423101) viability dye and then lyophilized. Dot plots above depict Zombie expression post lyophilization.

Figure 9. T, B and NK markers can be detected on mouse splenocytes post-lyophilization



Mouse splenocytes were isolated from a C57BL/6 mouse, processed and lyophilized. The resulting cells were reconstituted and stained with CD19, CD3 and CD49b.

Conclusions

1. The scatter patterns are preserved with our technology, allowing easy identification of lymphocytes and lymphocytes, monocytes and granulocytes.
2. Background fluorescence and most pattern and intensity staining performance metrics are equivalent to freshly prepared leukocytes.
3. The frequencies of CD3, CD4, CD8, CD16, CD56 positive cells are similar pre- and post-lyophilization, indicating excellent epitope preservation.
4. Veri-Cells™ products can be used to monitor reagent performance for most common surface molecules.
5. Robust expression of transcription factors such as Foxp3, T-bet and Helios was detected and mimicked that which is observed in fresh cells.
6. Intracellular molecules such as granzyme B and perforin expression can also be monitored.
7. Remarkable stability in closed vial and reconstituted conditions provides flexibility, reduced waste, and consistent reliability over long term experiments and trials.

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