R&D SYSTEMS
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Human Hematopoietic Progenitor Cell
Multi-Color Flow Cytometry Kit

Catalog Number: FMC019

Size: 25 Tests

PRODUCT DESCRIPTION

This kit contains four conjugated antibodies and four corresponding conjugated isotype controls for the identification of human hematopoietic progenitor cells (HPCs).

MATERIALS PROVIDED & STORAGE

Store the unopened kit at 2-8 °C **in the dark.** Refer to the kit label for date of expiration.

PART	PART #	DESCRIPTION
Positive Markers	967406	250 µL of CD34-APC Mouse IgG_1; Clone QBEnd10
	967409	250 µL of SCF R/CD117-PE Mouse IgG_1; Clone 47233
Negative Markers	967407	250 µL of CD38-PerCP Mouse IgG_{2A}; Clone 240742
	967408	250 µL of CD11b-CFS Mouse IgG_{2B}; Clone 238446
Positive Isotype Controls	965675	250 µL of Mouse IgG_1-APC Isotype Control; Clone 11711
	965666	250 µL of Mouse IgG_1-PE Isotype Control; Clone 11711
Negative Isotype Controls	967223	250 µL of Mouse IgG_{2A}-PerCP Isotype Control; Clone 20102
	967410	250 µL of Mouse IgG_{2B}-CFS Isotype Control; Clone 133303
Staining Buffer	895027	100 mL of 1X Staining Buffer

INTENDED USE

This product is designed for single-step, flow cytometric analysis of human HPCs using four fluorochrome-conjugated antibodies.

PRECAUTION

The Staining Buffer in this kit contains 0.09% sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

SURFACE STAINING PROTOCOL

1. Wash the cell samples with 2 mL of Staining Buffer, spinning the tube at 300 x g for 5 minutes.
2. Count the washed cells and then add Fc receptor blocking reagents if desired. If using excess pre-immune IgG to block Fc receptor, use 1 µg of IgG per 1 x 10^5 cells to be stained. The excess IgG does not need to be washed from the cells following the incubation period and can be carried into the staining reaction.
3. Transfer a small volume of cells (1 x 10^5 cells in 100 µL is recommended) into a 5 mL Flow Cytometry tube.
4. Add 10 µL of each positive antibody and 10 µL of each negative antibody to a tube of cells or add 10 µL of each corresponding isotype control antibody to a tube of cells.
5. Incubate the mixture for 30-45 minutes at room temperature **in the dark.**
6. Following the incubation, remove any excess antibody by washing the cells with 2 mL of Staining Buffer. Resuspend the final cell pellet in 200-400 µL of Staining Buffer for flow cytometric analysis.

   Note: *Using multiple fluorochromes requires proper flow cytometric compensation to remove the spillover fluorescence from a particular probe to a certain channel (1).*

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DATA EXAMPLES

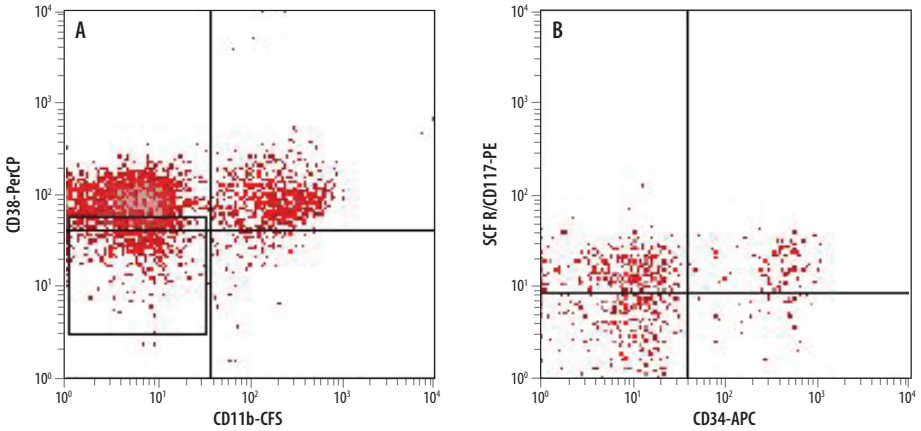

Figure 1: Human umbilical cord blood cells were stained using this kit. Cells negative for CD11b and negative/low for CD38 (boxed area in A) were gated and assessed for positive expression of CD34 and SCF R/CD117 (upper right quadrant in B). Quadrants were set based on isotype controls.

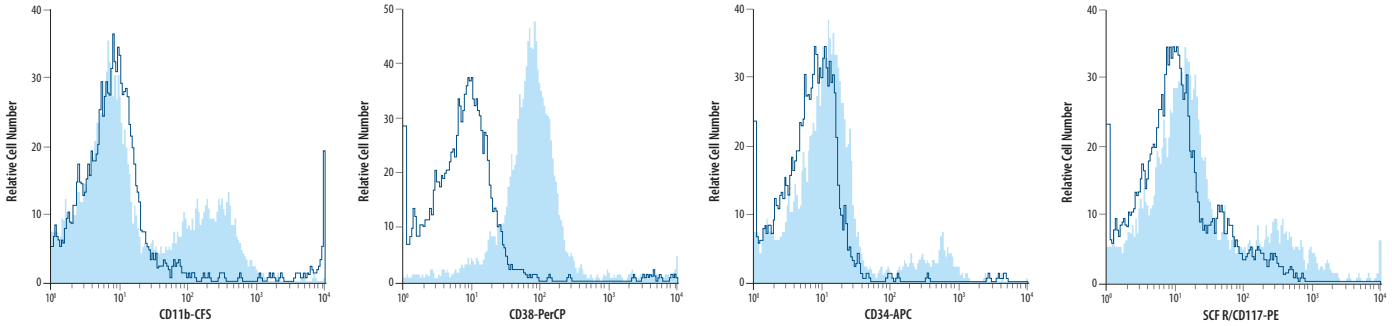

Figure 2: Human umbilical cord blood cells were stained with the indicated antibodies included in this kit. Representative expression of each analyte in the total umbilical cord blood cell population (filled histogram) over the isotype control (open histogram).

REFERENCES

1. Bagwell, B. and E.G. Adams (1993) Ann. N.Y. Acad. Sci. **677**:167.