

# Mouse Hematopoietic Progenitor Cell Multi-Color Flow Cytometry Kit

Catalog Number: FMC005 Size: 25 Tests

### Product Description

This kit contains three conjugated antibodies (and corresponding isotype controls) that can be used for single-step staining of mouse hematopoietic progenitor cells (mHPCs) (1 - 5):

- CD48-PE (Clone 331504; rat IgG<sub>1</sub>)
- CD150-APC (Clone 459911; rat IgG<sub>24</sub>)
- CD244-CFS (Affinity purified polyclonal antibody; goat IgG)

The kit also contains Staining Buffer (100 mL).

#### Intended Use

This product is designed for the flow cytometric analysis of mHPCs using three fluorochrome-conjugated antibodies.

#### Storage

Store at 2 - 8° C in the dark. Use within 6 months of receipt.

#### Precaution

The Staining Buffer contains 0.1% sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

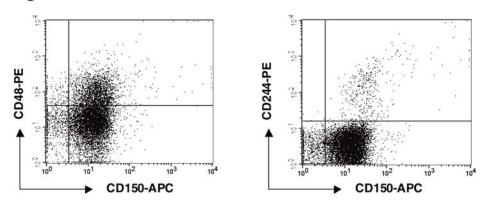
## Surface Staining Protocol

- 1. Cell samples should be washed with 2 mL of Staining Buffer, spinning the tube at 300 x g for 5 minutes.
- 2. Washed cells should be counted and then Fc receptor blocking reagents may be added. If using excess preimmune IgG to block Fc receptor, use 1  $\mu$ g 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 (about 100  $\mu$ L) of the Fc receptor-blocked cells (about 1 x 10 $^6$  cells) into a 5 mL Flow Cytometry tube.
- 4. Add 10  $\mu$ L of each antibody or each corresponding isotype control antibody to the cells.
- 5. Incubate the mixture for 30 45 minutes at 2 8° C in the dark.
- 6. Following the incubation, remove any excess antibody by washing the cells with 2 mL of Staining Buffer. The final cell pellet is resuspended in 200 400 μL of Staining Buffer for flow cytometric analysis.

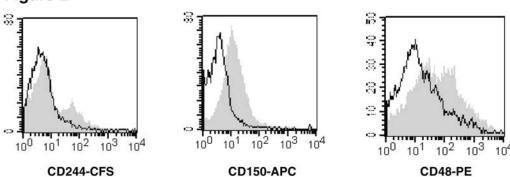
**Note:** Using multiple fluorochromes requires proper flow cytometric compensation to remove the spillover fluorescence from a particular probe to a certain channel (6).

## Typical Data

## Figure 1







**Figure 1:** Staining of mouse bone marrow cells with the indicated antibodies (quandrants set with the corresponding isotype controls).

**Figure 2:** Staining of mouse splenocytes with the indicated antibodies (filled histograms) or the corresponding isotype control (open histograms).

## References

- 1. Kiel, M.J. et al. (2005) Cell 121:1109.
- 2. Purton, L.E. and D.T. Scadden (2007) Cell Stem Cell 1:263.
- 3. Yilmaz, O.H. et al. (2006) Blood 107:924.
- 4. Kim, I. et al. (2006) Blood 108:737.
- 5. Lai, A.Y. and M. Kondo (2008) Sem. Immunol. 20:207.
- 6. Bagwell, B. and E.G. Adams (1993) Ann. N.Y. Acad. Sci. 677:167.

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