APC-Cy™7 Rat Anti-Mouse Ly-6A/E

**Product Information**

Material Number: 560654
Alternate Name: Ly-6A/E; Lymphocyte antigen 6A-2/6E-1; Ly-6A.2/Ly-6E.1; Sca-1; TAP
Size: 50 µg
Concentration: 0.2 mg/ml
Clone: D7
Immunogen: IL-2-dependent mouse T-cell line CTL-L
Isotype: Rat (LEW) IgG2a, κ
Reactivity: Mouse
Storage Buffer: Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

**Description**

The D7 monoclonal antibody recognizes Ly-6A.2 and Ly-6E.1, which are allelic members of the Ly-6 multigene family. Sca-1 (Ly6A/E), a phosphatidylinositol-anchored protein of about 18 kDa, is expressed on the multipotent hematopoietic stem cells (HSC) in the bone marrow of mice with both Ly-6 haplotypes. In mice expressing the Ly-6.2 haplotype (e.g., AKR, C57BL, C57BR, C57L, C58, DBA/2, PL, SJL, SWR, 129), Ly-6A/E is also expressed on distinct subpopulations of bone marrow and peripheral B lymphocytes as well as thymic and peripheral T lymphocytes. Strains with the Ly-6.1 haplotype (e.g., A, BALB/c, CBA, C3H/He, DBA/1, NZB) have few Ly-6A/E+ resting peripheral lymphocytes; activation of lymphocytes from mice of both Ly-6 haplotypes leads to strong expression of the Sca-1 antigen. Studies with the D7 antibody have demonstrated that Ly-6A/E may be involved in the regulation of B and T lymphocyte responses, and appears to be required for T-cell receptor-mediated T-cell activation. The purified E13-161.7 mAb (anti-Ly-6A/E) can block binding of FITC-conjugated D7 antibody to mouse splenocytes, but purified mAb D7 is unable to block binding of FITC-conjugated E13-161.7 antibody. Anti-Ly-6A/E (Sca-1) mAb may be used in combination with a Mouse Lineage Panel of antibodies to identify HSC.

**Preparation and Storage**

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with APC-Cy7 under optimum conditions, and unconjugated antibody and free APC-Cy7 were removed.

**Application Notes**

Application

- Flow cytometry Routinely Tested

**Suggested Companion Products**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tr>
<td>552770</td>
<td>APC-Cy™7 Rat IgG2a, κ Isotype Control</td>
<td>0.1 mg</td>
<td>R35-95</td>
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<td>554656</td>
<td>Stain Buffer (FBS)</td>
<td>500 mL</td>
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<tr>
<td>554657</td>
<td>Stain Buffer (BSA)</td>
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</tbody>
</table>

Flow cytometric analysis of Ly-6A/E on stimulated mouse splenocytes. BALB/c splenocytes were stained with 2.5-5.0 µg/mL concanavalin A (Sigma-Aldrich cat. no. C2010) for 48 hours and were subsequently stained either with a APC-Cy™7 Rat IgG2a, κ isotype control (Cat. No. 552770, unshaded) or with the APC-Cy™7 Rat Anti-Mouse Ly-6A/E antibody (Cat. No. 560654, shaded). Histograms were derived from gated events based on light scattering characteristics for splenocytes. Flow cytometry was performed on a BD™ LSR II flow cytometry system.
Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
5. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
6. APC-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher.
7. APC-Cy7 is a tandem fluorochrome composed of Allophycocyanin (APC), which is excited by laser lines between 595 and 647 nm and serves as an energy donor, coupled to the cyanine dye Cy7™, which acts as an energy acceptor and fluoresces at 780 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in APC-Cy7, thus maximizing its fluorescence emission intensity, minimizing residual emission from APC, and minimizing required electronic compensation in multilaser-laser flow cytometry systems. Note: Although every effort is made to minimize the lot-to-lot variation in residual emission from APC, it is strongly recommended that every lot be tested for differences in the amount of compensation required and that individual compensation controls are run for each APC-Cy7 conjugate.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
9. Cy is a trademark of GE Healthcare.

References

Jurecic R, Van NT, Belmont JW. Enrichment and functional characterization of Sca-1+WG-1, Lin-WGA-, Lin-Sca-1+, and Lin-Sca-1+WG-1 bone marrow cells from mice with an Ly-6a haplotype. Blood. 1993; 82(9):2673-2683. (Biology)
Spangrude GJ, Brooks DM. Mouse strain variability in the expression of the hematopoietic stem cell antigen Ly-6A/E by bone marrow cells. Blood. 1993; 82(11):3327-3332. (Biology)
vanderWijn M, Heimfeld S, Spangrude GJ, Weissman IL. Mouse hematopoietic stem-cell antigen Sca-1 is a member of the Ly-6 antigen family. Proc Natl Acad Sci U S A. 1989; 86(12):4634-4638. (Biology)