Technical Data Sheet

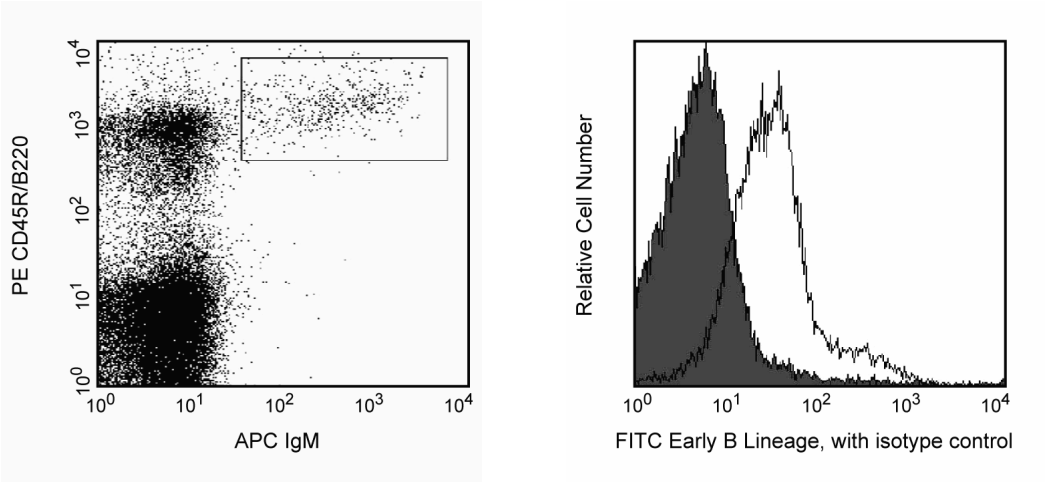
FITC Rat Anti-Mouse Early B Lineage

Product Information

Material Number:	**559156**
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	AA4.1
Immunogen:	Pre-B lymphoma 70Z/3, derived from (C57BL/6 x DBA/2)F1 mouse
Isotype:	Rat (SD) IgG2b, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The AA4.1 antibody reacts with a 130-140-kDa type I transmembrane protein expressed on immature B lymphocytes in the adult bone marrow; and on hematopoietic progenitors and stem cells in adult bone marrow, fetal liver, and embryonic yolk sac. Although staining of splenic immature/transitional B cells has been reported, we find that the antigen density is much lower in the spleen than in the bone marrow. Staining of spleen requires amplification through the use of a second step. The FITC conjugate of mAb AA4.1, while ideal for bone marrow staining, is not effective in the spleen (see Reccomended Assay Procedure below). It has been observed that the staining pattern of mAb 493 (Cat. Nos. 550433 and 550434 for the purified and biotinylated formats, respectively) is similar to that of mAb AA4.1, that both antibodies precipitate molecules of the same molecular weight, and that staining by mAb AA4.1 is not blocked by mAb 493, suggesting that the antibodies recognize separate epitopes of the same Early B Lineage antigen.



Three-color analysis of the expression of the Early B Lineage antigen in mouse bone marrow. *BALB/c bone marrow leukocytes were simultaneously stained with PE Rat anti-Mouse CD45R/B220(Cat. No. 553089/553090), APC Rat Anti-Mouse IgM (Cat. No. 550676), and either FITC Rat IgG2b, κ isotype control (Cat. No. 553988) or FITC Rat anti-Mouse Early B Lineage mAb. Live cells were selected by exclusion of propidium iodide. Double-positive IgM+ CD45R+ cells were gated (left panel) and analyzed for FITC signal. In the right panel, the solid histogram represents the isotype control, while the open histogram represents the expression of the Early B Lineage antigen. Approximately 70% of the IgM+ CD45R+ bone marrow cells are also AA4.1 positive. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

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 FITC under optimum conditions, and unreacted FITC was removed.

Application Notes

Application

Flow cytometry	Routinely Tested

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

BD, BD Logo and all other trademarks are the property of Becton, Dickinson and Company. ©2011 BD

Recommended Assay Procedure:

For detection of the Early B Lineage antigen in the spleen, we recommend amplification of the staining signal through the use of biotinylated mAb 493 (Cat. No. 550434), followed by a "bright" second-step reagent, such as Streptavidin-PE (Cat. No. 554061).

Suggested Companion Products

Catalog Number	Name	Size	Clone
553089	PE Rat Anti-Mouse CD45R/B220	0.1 mg	RA3-6B2
550676	APC Rat Anti-Mouse IgM	0.1 mg	II/41
553988	FITC Rat IgG2b, κ Isotype Control	0.25 mg	A95-1

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. An isotype control should be used at the same concentration as the antibody of interest.

References

Allman D, Li J, Hardy RR. Commitment to the B lymphoid lineage occurs before DH-JH recombination. *J Exp Med.* 1999; 189(4):735-740. (Biology)

Allman D, Lindsley RC, DeMuth W, Rudd K, Shinton SA, Hardy RR. Resolution of three nonproliferative immature splenic B cell subsets reveals multiple selection points during peripheral B cell maturation. *J Immunol.* 2001; 167(12):6834-6840. (Biology)

Auerbach R, Huang H, Lu L. Hematopoietic stem cells in the mouse embryonic yolk sac. *Stem Cells.* 1996; 14(3):269-280. (Biology)

Jordan CT, McKearn JP, Lemischka IR. Cellular and developmental properties of fetal hematopoietic stem cells. *Cell.* 1990; 61(6):953-963. (Biology)

Lacaud G, Carlsson L, Keller G. Identification of a fetal hematopoietic precursor with B cell, T cell, and macrophage potential. *Immunity.* 1998; 9(6):827-838. (Biology)

Li YS, Wasserman R, Hayakawa K, Hardy RR. Identification of the earliest B lineage stage in mouse bone marrow. *Immunity.* 1996; 5(6):527-535. (Biology)

McKearn JP, Baum C, Davie JM. Cell surface antigens expressed by subsets of pre-B cells and B cells. *J Immunol.* 1984; 132(1):332-339. (Immunogen)

Paige CJ, Gisler RH, McKearn JP, Iscove NN. Differentiation of murine B cell precursors in agar culture. Frequency, surface marker analysis and requirements for growth of clonable pre-B cells. *Eur J Immunol.* 1984; 14(11):979-987. (Biology)

Szilvassy SJ, Cory S. Phenotypic and functional characterization of competitive long-term repopulating hematopoietic stem cells enriched from 5-fluorouracil-treated murine marrow. *Blood.* 1993; 81(9):2310-2320. (Biology)