

Technical Data Sheet

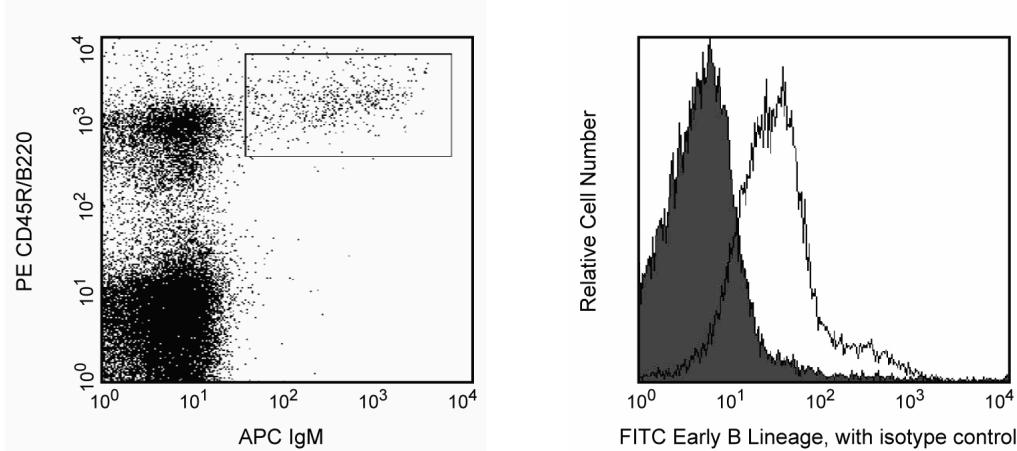
FITC Rat Anti-Mouse Early B Lineage

Product Information

Material Number:	561990
Size:	50 µg
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 Recommended 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
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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).

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## 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, $\kappa$ 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/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/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](http://www.bdbiosciences.com/colors).

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