Technical Data Sheet

BV421 Rat Anti-Mouse CD34

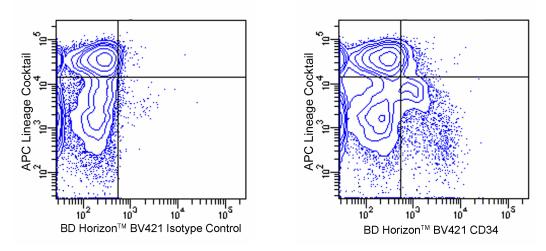
Product Information

Material Number:	562608
Alternate Name:	Cd34; CD34 antigen; Hematopoietic progenitor cell antigen CD34
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	RAM34
Immunogen:	Recombinant Mouse CD34
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ${\leq}0.09\%$ sodium azide.

Description

The RAM34 specifically binds to the CD34 glycoprotein on the surface of three independently derived mouse CD34-transfected cell lines. RAM34 antibody also reacts with the mouse cell lines PA6, 416B, Swiss 3T6, NIH, 3T3, DA1, and M1, all of which are positive for expression of mouse CD34 mRNA. Cell lines shown to be negative for CD34 transcript, including WEHI-3B, EL4, 18.8, and CMT64/61, are also negative for surface expression of CD34 as determined by RAM34 staining. Normal thymocytes and splenocytes are negative for CD34 expression. In the bone marrow, 7-10% of cells are stained with RAM34 mAb, including most of the Ly-6A/E (Sca-1)+ CD90 (Thy-1)low Lineage Marker- hematopoietic stem cell-enriched subpopulation and myeloerythroid progenitors. CD34 is also expressed on a small percentage of fetal liver cells, including NK-cell progenitors. CD34 has been reported to be expressed on the endothelium of capillaries and, in this form, to function as a ligand for L-selectin. Consistent with this observation, RAM34 antibody stains endothelial cells in spleen, thymus, and postcapillary HEVs in the lymph nodes. It is reported that RAM34 antibody can be used to select CD34+ CD117 (c-Kit)+ Ly-6A/E (Sca-1)+ Lineage Marker- bone marrow-derived hematopoietic stem cells, capable of short-term multi-lineage reconstitution of lethally irradiated mice; while the CD34- CD117+ Sca-1+ Lineage Marker- population contains self-renewing hematopoietic stem cells. Similarly, the bone marrow population with high dye-efflux capacity and which is highly enriched for long-term reconstituting hematopoietic stem cells is CD34- CD117 (c-Kit)+ Ly-6A/E (Sca-1)+ Lineage Marker-.

The antibody was conjugated to BD Horizon™ BV421 which is part of the BD Horizon™ Brilliant Violet™ family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD HorizonTM BV421 can be excited by the violet laser and detected in the standard Pacific BlueTM filter set (eg, 450/50-nm filter). BD Horizon™ BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific BlueTM conjugates.



Multicolor flow cytometric analysis of CD34 expression on mouse bone marrow cells. BALB/c bone marrow cells were treated with Mouse BD Fc Block™ [Purified Rat Anti-Mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/ 553142)] and stained with either BD Horizon™ BV421 Rat IgG2a, κ Isotype Control (Left Panel) or BD Horizon™ BV421 Rat Anti-Mouse CD34 antibody (Right Panel) followed by staining with APC Mouse Lineage Antibody Cocktail (Cat. No. 558074) to identify major lineage-committed cell types. Two-color flow cytometric contour plots were derived from gated events with the forward and side light-scatter characteristics of viable bone marrow leukocvtes. Flow cvtometry was performed using a BD FACSCanto™ II Flow Cvtometer Svstem.

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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 BD HorizonTM BV421 under optimum conditions, and unconjugated antibody and free BD HorizonTM

BV421 were removed.

Application Notes

Application

Flow cytometry Routinely Tested

Recommended Assay Procedure:

Flow Cytometry: Investigators are highly encouraged to titrate this reagent for optimal results as individual experimental systems may vary. A starting concentration of 2 µg/mL is recommended.

Suggested Companion Products

Catalog Number	Name	Size	Clone	
554656	Stain Buffer (FBS)	500 ml	(none)	
562602	BV421 Rat IgG2a, κ Isotype Control	50 µg	R35-95	
558074	APC Mouse Lineage Antibody Cocktail, with Isotype Control	100 tests	(none)	
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block TM)	0.1 mg	2.4G2	
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2	

Product Notices

Since applications vary, each investigator should titrate the reagent to obtain optimal results. 1.

- 2. An isotype control should be used at the same concentration as the antibody of interest.
- Brilliant Violet[™] 421 is a trademark of Sirigen. 3.
- 4. Pacific Blue[™] is a trademark of Molecular Probes, Inc., Eugene, OR.
- 5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before 6. discarding to avoid accumulation of potentially explosive deposits in plumbing.
- 7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
- 8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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