Technical Data Sheet

Purified Mouse Anti-Rat CD32

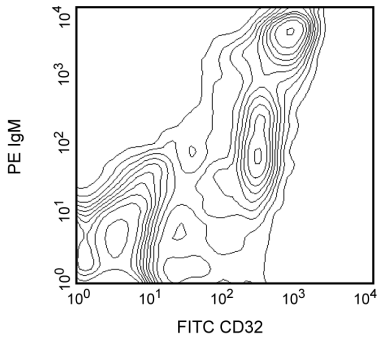
Product Information

Material Number:	**550270**
Alternate Name:	FcγII Receptor
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	D34-485
Immunogen:	Recombinant Rat CD32 Protein
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Rat
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

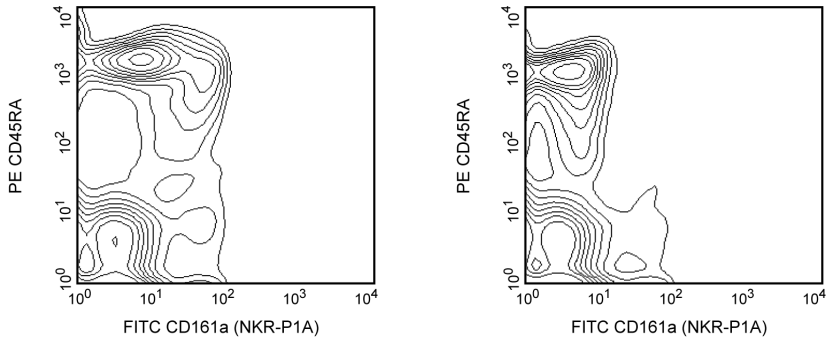
Description

The D34-485 antibody reacts specifically with CD32, the FcγII receptor. Rat CD32 is expressed on B lymphocytes, myeloid cells, and some lymphocytes in the thymic medulla. D34-485 mAb blocks binding of aggregated immunoglobulins to the FcγIi receptors in vitro.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Two-color analysis of the expression of Cd32 on rat splenocytes. *Lewis rat splenocytes were stained with purified D34-485 mAb, followed by FITC-conjugated polyclonal goat anti-mouse Ig (Cat. No. 554001), then PE-conjugated anti-rat IgM G35-238 (Cat. No. 553888). Double-positive cells are B lymphocytes (IgM+ cells), which express CD32 on the cell surface. Flow cytometry was performed on a BD FACScan™ flow cytometry system.*



Blocking of Fc-mediated binding to FcγII receptors (CD32) on rat splenocytes. *Lewis rat splenocytes were pre-incubated with purified isotype control mAb A112-2 (Cat. No. 553487, left panel) or Rat BD Fc Block™ purified anti-rat CD32 mAb D34-485 (right panel). Two-color staining was performed with FITC-conjugated anti-rat NKR-P1A mAb 10/78 (Cat. No. 555008) and PE-conjugated anti-rat CD45RA mAb OX-33 (Cat. No. 551402/554884). Note how the dim staining of B lymphocytes (OX-33+ cells) by anti-CD161a (NKR-P1A) (left panel) is reduced when Rat BD Fc Block™ is used before staining (right panel). Flow cytometry was performed on a BD FACScan™ flow cytometry system.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

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Application Notes

Application

Flow cytometry	Routinely Tested
Immunohistochemistry-frozen	Reported
Immunohistochemistry-zinc-fixed	Reported
Western blot	Reported
Blocking	Reported
Immunohistochemistry-paraffin	Not Recommended

Recommended Assay Procedure:

To specifically stain cells bearing FcγII receptors for flow cytometric analysis: Incubate cell suspension with this antibody (≤ 1 µg/million cells) followed by an appropriate fluorochrome-conjugated second-step reagent.

To reduce Fc receptor-mediated binding by antibodies of interest to FcγII receptor-bearing rat cells for flow cytometric analysis:

a. Preincubate cell suspension with Rat BD Fc Block™ (e.g., ≤ 1 µg/million cells in 100 µl) at 4°C for 5 minutes.

b. Add antibody of interest directly to preincubated cells in the presence of Rat BD Fc Block™ (i.e., Rat BD Fc Block™ need not be washed off before staining cells)

c. If anti-Ig second-step is necessary, a reagent must be chosen which will not bind to Rat BD Fc Block™ (e.g., mouse IgG1, κ)

Suggested Companion Products

Catalog Number	Name	Size	Clone
555988	FITC Goat Anti-Mouse IgG/IgM	0.5 mg	Polyclonal
554001	FITC Goat Anti-Mouse Igs	0.5 mg	Polyclonal
557273	Purified Mouse IgG1, κ Isotype Control	0.5 mg	MOPC-31C
553888	PE Mouse Anti-Rat IgM	0.2 mg	G53-238
551402	PE Mouse Anti-Rat CD45RA	0.1 mg	OX-33
555008	FITC Mouse Anti-Rat CD161a	0.5 mg	10/78

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. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE™ (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.