Technical Data Sheet

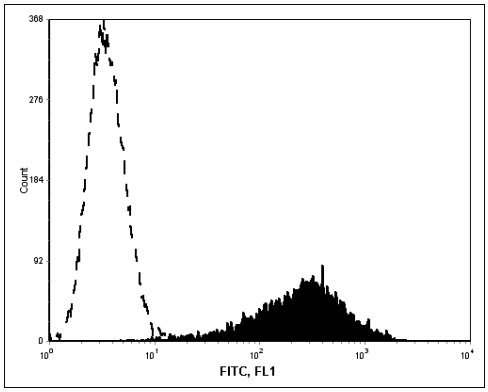
Purified Mouse Anti-Rat Mast Cells

Product Information

Material Number:	**551770**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	AR32AA4 (also known as AA4)
Immunogen:	Rat basophilic leukemia cell line
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Rat
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The AR32AA4 (AA4) antibody reacts with two distinct α-galactosyl derivatives of the ganglioside GD1b (disialogangliosides). The mAb AA4 reacts with four indistinct bands ranging in size from 56 - 110 kDa. Binding of the AA4 antibody to RBL-2H3 cells has been shown to inhibit IgE-mediated histamine release by inhibiting FcεR1 signal transduction events. The mAb AA4 has been shown to be useful for immunomagnetic mast cell separation from rat bone marrow and peritoneal lavage cell populations. This corresponds with reports that the AA4-binding epitope is expressed at higher levels than the FcεR1 on RBL-2H3 cells.



Staining of mAb AR32AA4 on RBL (ATCC CRL-2256) cells. *RBL cells were incubated with either Purified Mouse IgG1, κ isotype control (Cat. no. 557273, open dash line overlay) or purified AR32AA4 mAb (shaded histogram), followed by biotinylated F(ab')2 rat antimouse IgG and finally Streptavidin-FITC (Cat. no. 554060). The cells in both the shaded histogram and the overlay were gated on 7-AAD negative cells. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Preparation and Storage

Store undiluted at 4°C.

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

Application Notes

Application

Flow cytometry	Routinely Tested
Cell separation	Reported
Immunohistochemistry	Reported
Immunoprecipitation	Reported
Inhibition	Reported
Immunocytochemistry (cytospins)	Reported

Recommended Assay Procedure:

It is recommended that for immunofluorescent staining of rat cells, the AA4 antibody be carefully titrated and used with F(ab')2 secondary reagents, such as a biotinlayted F(ab')2 rat anti-mouse IgG (multiple adsorption).

Suggested Companion Products

Catalog Number	Name	Size	Clone
557273	Purified Mouse IgG1, κ Isotype Control	0.5 mg	MOPC-31C
554060	FITC Streptavidin	0.5 mg	(none)
554970	PE Mouse Anti-Rat CD54	0.2 mg	1A29
554656	Stain Buffer (FBS)	500 ml	(none)

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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. 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.
4. 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.
5. An isotype control should be used at the same concentration as the antibody of interest.

References

Basciano LK, Berenstein EH, Kmak L, Siraganian RP. Monoclonal antibodies that inhibit IgE binding. *J Biol Chem.* 1986; 261(25):11823-11831. (Immunogen: Immunoprecipitation, Inhibition)

Faraco CD, Vugman I, Siraganian RP, Jamur MC, Oliver C. Immunocytochemical identification of immature rat peritoneal mast cells using a monoclonal antibody specific for rat mast cells. *Acta Histochem Suppl.* 1997; 99(1):23-27. (Clone-specific: Immunocytochemistry (cytospins))

Guo NH, Her GR, Reinhold VN, Brennan MJ, Siraganian RP, Ginsburg V. Monoclonal antibody AA4, which inhibits binding of IgE to high affinity receptors on rat basophilic leukemia cells, binds to novel alpha-galactosyl derivatives of ganglioside GD1b. *J Biol Chem.* 1989; 264(22):13267-13272. (Biology)

Jamur MC, Grodzki AC, Moreno AN, de Mello Lde F, Pastor MV. Identification and isolation of rat bone marrow-derived mast cells using the mast cell-specific monoclonal antibody AA4. *J Histochem Cytochem.* 2001; 49(2):219-228. (Clone-specific: Cell separation)

Jamur MC, Grodzki AC, Moreno AN, Swaim WD, Siraganian RP, Oliver C. Immunomagnetic isolation of rat bone marrow-derived and peritoneal mast cells. *J Histochem Cytochem.* 1997; 45(12):1715-1722. (Clone-specific: Cell separation)

Stephan V, Seibt A, Dukanovic D, Skasa M. Anti-ganglioside monoclonal antibody AA4 selectively inhibits IgE-induced signal transduction pathways in rat basophilic leukemia cells. *Mol Immunol.* 1997; 34(3):227-235. (Clone-specific: Inhibition)