

Technical Data Sheet

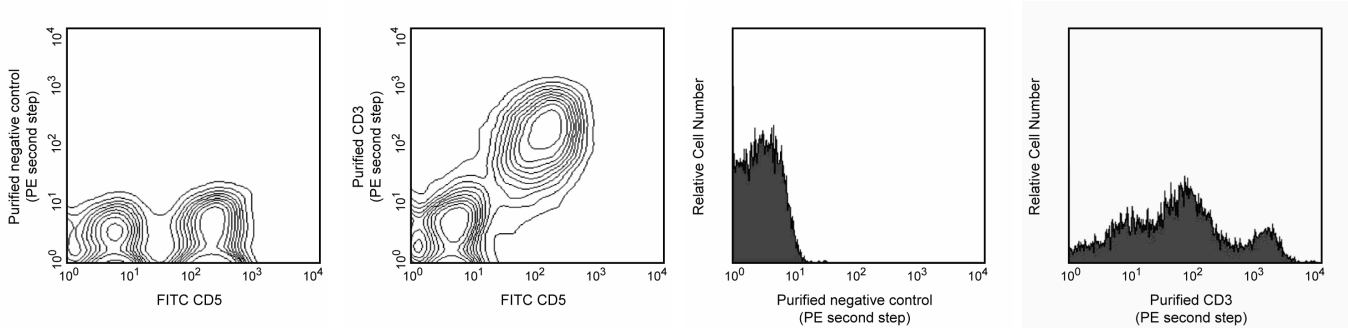
Purified Mouse Anti-Rat CD3

Product Information

Material Number:	556970
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	1F4
Immunogen:	F344 rat splenic T cells stimulated with PMA plus A23187
Isotype:	Mouse (BALB/c) IgM, κ
Reactivity:	QC Testing: Rat
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 1F4 antibody reacts with the T-cell receptor-associated CD3 cell-surface antigen found on thymocytes, peripheral T lymphocytes, and dendritic epidermal T cells. In vitro, immobilized 1F4 mAb induces T-cell proliferation, and soluble mAb synergizes with PMA for T-cell activation. Pre-incubation of splenocytes with mAb 1F4 blocks staining with mAb G4.18 (Cat. No. 559974/554830). 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.



CD3e expression in spleen and thymus. LEW splenocytes were preincubated with Rat BD Fc Block™ purified anti-rat CD32 mAb D34-485 (Cat. No. 550270/550271) and simultaneously stained with FITC-conjugated anti-rat CD5 mAb OX-19 (Cat. No. 551449, left panels) and purified mAb 1F4 (middle left panel), followed by PE-conjugated anti-mouse IgMa mAb DS-1 (Cat. No. 553517, left panels). LEW thymocytes were stained with purified 1F4 mAb (far right panel) followed by PE-conjugated anti-mouse IgMa mAb DS-1 (right panels). Flow cytometry was performed on a BD FACSCalibur™ 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.

Application Notes

Application

Flow cytometry	Routinely Tested
Cytotoxicity	Reported
Immunoprecipitation	Reported
Immunohistochemistry-frozen	Reported
Fluorescence microscopy	Reported
Stimulation	Reported

Recommended Assay Procedure:

We recommend FITC- or PE-conjugated anti-mouse IgMa mAb DS-1 (Cat. No. 553516 or 553517, respectively) as a secondary antibody for immunofluorescent staining because we have found that polyclonal anti-mouse Ig antibodies may lose much of their reactivity to mouse IgM after adsorption with rat Ig. For IHC, we recommend the use of purified G4.18 mAb in our special formulation for immunohistochemistry, Cat. No. 550295. Although mAb 1F4 is reported to be effective for in vitro T-cell stimulation, we have found that the alternate anti-rat CD3 antibody G4.18 (Cat. no. 554829), soluble or immobilized, has greater stimulatory activity.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
553472	Purified Mouse IgM, κ Isotype Control	0.5 mg	G155-228
555988	FITC Goat Anti-Mouse IgG/IgM	0.5 mg	Polyclonal

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmlingen/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.

References

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Naper C, Vaage JT, Lambricht D, et al. Alloreactive natural killer cells in the rat: complex genetics of major histocompatibility complex control. *Eur J Immunol.* 1995; 25(5):1249-1256.(Biology)

Nicolls MR, Aversa GG, Pearce NW, et al. Induction of long-term specific tolerance to allografts in rats by therapy with an anti-CD3-like monoclonal antibody. *Transplantation.* 1993; 55(3):459-468.(Clone-specific: Cytotoxicity)

Tanaka, T., Masuko, T., Yagita, H. et al.. Characterization of a CD3-like rat T cell surface antigen recognized by a monoclonal antibody.. *J Immunol.* 1989; 142:2791-2795.(Immunogen: Immunohistochemistry, Immunoprecipitation, Stimulation)

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