Technical Data Sheet

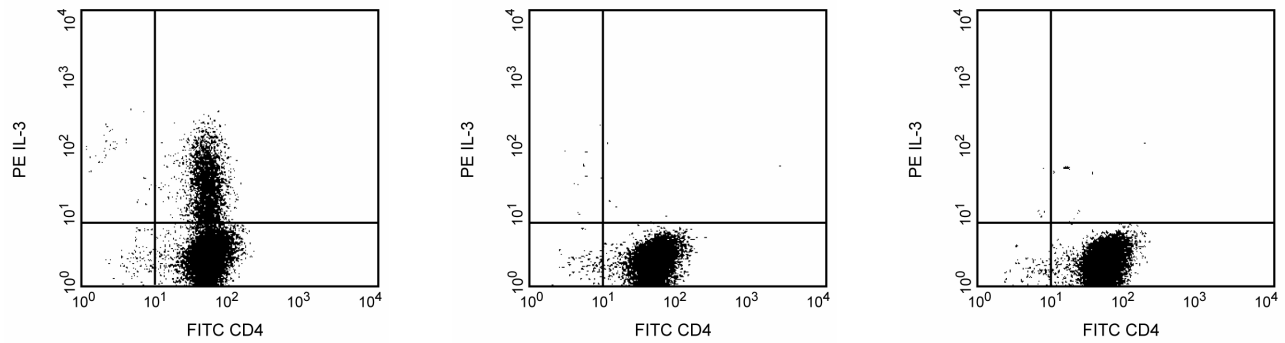
PE Rat Anti-Mouse IL-3

Product Information

Material Number:	**554383**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	MP2-8F8
Immunogen:	Recombinant mouse IL-3
Isotype:	Rat IgG1
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The MP2-8F8 antibody reacts with mouse interleukin-3 (IL-3). The immunogen used to generate the MP2-8F8 hybridoma was recombinant mouse IL-3. This is a neutralizing antibody.



Expression of IL-3 by stimulated CD4+ Balb/c spleen cells. *Purified splenic CD4+ cells from 6 month old BALB/c mice were stimulated with plate-bound anti-CD3 (25 µg/ml final concentration; 145-2C11, Cat. No. 553057) and soluble anti-mouse CD28 (2 µg /ml final concentration; clone 37.51, Cat. No. 553294) for 2 days in culture together with recombinant mouse IL-2 (10 ng/ml final concentration; Cat. No. 550069) and recombinant mouse IL-4 (0.5 ng/ml final concentration; Cat. No. 550067), followed by a 3 day incubation with only recombinant mouse IL-2 and recombinant mouse IL-4. This was followed by a 5 hour stimulation with plate-bound anti-CD3 (25 µg/ml final concentration) and anti-mouse CD28 (2 µg/ml final concentration) in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). The cells were harvested, stained with 0.06 µg of FITC-conjugated rat anti-mouse CD4 (FITC-RM4-5, Cat. No. 553047), fixed, permeabilized, and subsequently stained with 0.12 µg of PE-conjugated rat anti-mouse IL-3 antibody (PE-MP2-8F8, Cat. No. 554383) by using the BD Pharmingen staining protocol (left panel). To demonstrate specificity of staining, the binding by PE-MP2-8F8 was blocked by each of the following: 1) preincubation of the conjugated antibody with molar excess of recombinant mouse IL-3 (0.12 µg, Cat. No. 554579; center panel) and by 2) preincubation of the fixed/permeabilized cells with excess unlabeled MP2-8F8 mouse antibody (3 µg; Cat. No. 554380; right panel) prior to staining with the PE-MP2-8F8. The quadrant markers for the bivariate dot plots were set based on the autofluorescence controls and verified using the recombinant cytokine blocking and unlabeled antibody blocking specificity controls.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.
Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The MP2-8F8 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-3 producing cells within mixed cell populations. PE-conjugated MP2-8F8 antibody (Cat. No. 554383) is especially suitable for these studies (see Figure, left panel). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.25 µg mAb/million cells). For specific methodology, please visit the protocols section or chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

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A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the conjugated MP2-8F8 antibody with a molar excess of ligand (e.g., recombinant mouse IL-3; Cat No. 554579) prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabeled MP2-8F8 antibody (Cat. No. 554380, 554381) prior to staining. The staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable rat IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse or human cells is PE-R3-34 (Cat. No. 554685); use at comparable concentrations to antibody of interest (e.g., ≤ 0.25 µg mAb/1 million cells).

Neutralization: The NA/LE™ MP2-8F8 antibody (Cat. No. 554379) is useful for neutralization of mouse IL-3 bioactivity.

ELISA Capture: The purified MP2-8F8 antibody (Cat. No. 554380) is useful as a capture antibody for a sandwich ELISA for measuring mouse IL-3 protein levels. For specific methodology, please visit the protocols section or chapter on ELISA in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com. For testing mouse IL-3 in complex biological fluids such as serum or plasma, our mouse IL-3 specific OptEIA™ sandwich ELISA set is recommended (Cat. No. 555228).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554685	PE Rat IgG1, κ Isotype Control	0.1 mg	R3-34
553057	Purified NA/LE Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
553294	Purified NA/LE Hamster Anti-Mouse CD28	0.5 mg	37.51
550069	Recombinant mouse IL-2	20 µg	(none)
550067	Recombinant Mouse IL-4	10 µg	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554653	Mick-2 Cytokine Positive Control Cells	NA	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
555228	Mouse IL-3 ELISA Set	20 plates	(none)

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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.
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.

References

Abrams J. Immunoenzymetric assay of mouse and human cytokines using NIP-labeled anti-cytokine antibodies. In: Coligan J, Kruisbeek A, Margulies D, Shevach E, Strober W, ed. *Current Protocols in Immunology*. New York: John Wiley and Sons; 1995:6.20-6.21.(Clone-specific: ELISA)

Abrams JS, Pearce MK.. Development of rat anti-mouse interleukin 3 monoclonal antibodies which neutralize bioactivity in vitro. *J Immunol.* 1988; 140(1):131-137. (Clone-specific: ELISA, Neutralization)

Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24.(Clone-specific: ELISA, Neutralization)

Cockayne DA, Muchamuel T, Grimaldi JC, et al. Mice deficient for the ecto-nicotinamide adenine dinucleotide glycohydrolase CD38 exhibit altered humoral immune responses. *Blood.* 1998; 92(4):1324-1333.(Clone-specific: ELISA, Neutralization)

Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods.* 1995; 188(1):117-128.(Methodology: IC/FCM Block)

Sander B, Hoiden I, Andersson U, Moller E, Abrams JS. Similar frequencies and kinetics of cytokine producing cells in murine peripheral blood and spleen. Cytokine detection by immunoassay and intracellular immunostaining. *J Immunol Methods.* 1993; 166(2):201-214.(Clone-specific: ELISA)