Technical Data Sheet

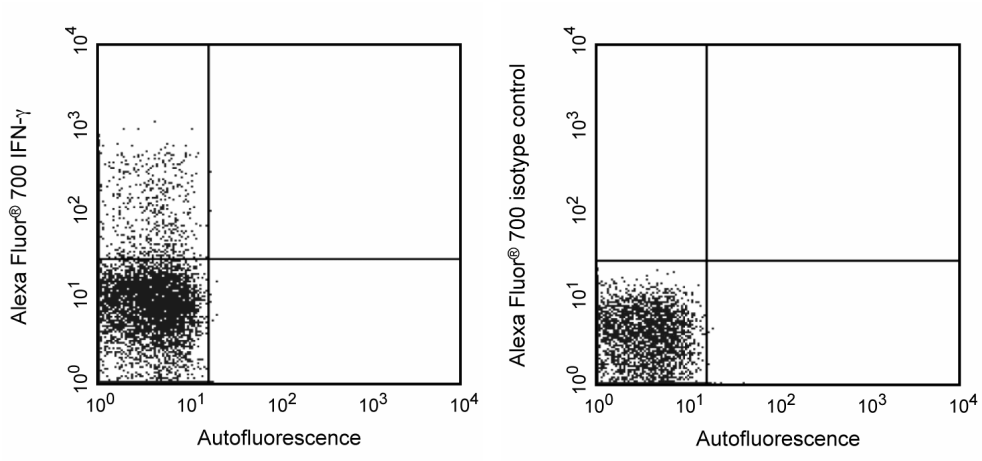
Alexa Fluor® 700 Mouse Anti-Human IFN-γ

Product Information

Material Number:	**561024**
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	B27
Immunogen:	Human IFN-γ Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The B27 antibody reacts with human interferon-γ (IFN-γ) and reported not to bind to denatured IFN-γ.



Expression of IFN-γ by stimulated human peripheral blood lymphocytes (PBMCs). *Human PBMCs were stimulated with PMA and ionomycin in the presence of Brefeldin A. The cells were harvested, fixed, permeabilized, and stained with either Alexa Fluor® 700 Mouse Anti-Human IFN-γ (left panel) or Alexa Fluor® 700 Mouse IgG1, κ Isotype Control mAb MOPC-21 (Cat. No. 557882, right panel) according to the Recommended Assay Procedure. To demonstrate the specificity of staining, the binding of the Alexa Fluor® 700 conjugate of mAb B27 was blocked by preincubation of the fixed/permeabilized cells with an excess of unlabeled B27 mAb (10 µg, Cat. No. 554699, data not shown) prior to staining. The quadrant markers for the bivariate dot plots were set based on the autofluorescence and isotype controls.*

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 to Alexa Fluor® 700 under optimum conditions, and unreacted Alexa Fluor® 700 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Flow Cytometry: The Alexa Fluor® 700-conjugated B27 antibody (Cat. No. 557995) antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IFN-γ producing cells within mixed cell populations. For optimal immunofluorescent staining for flow cytometric analysis, the 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. A useful control for demonstrating specificity of staining is the unlabeled B27 antibody used to pre-block the fixed/permeabilized cells (Cat. No. 554699) prior to staining. A suitable mouse IgG1 isotype control for assessing the level of background staining is Alexa Fluor® 700-conjugated mAb MOPC-21 (Cat. No. 557882).

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Suggested Companion Products

Catalog Number	Name	Size	Clone
557882	Alexa Fluor® 700 Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554699	Purified Mouse Anti-Human IFN-γ	0.1 mg	B27
555061	HiCK-1 Human Cytokine Positive Control Cells	1.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Alexa Fluor® 700 has an adsorption maximum of ~700nm and a peak fluorescence emission of ~720nm. Before staining cells with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
3. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
4. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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)

Favre C, Wijdenes J, Cabrillat H, Djossou O, Banchereau J, de Vries JE. Epitope mapping of recombinant human gamma interferon using monoclonal antibodies. *Mol Immunol.* 1989; 26(1):17-25. (Clone-specific: Immunoprecipitation, 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)