Technical Data Sheet

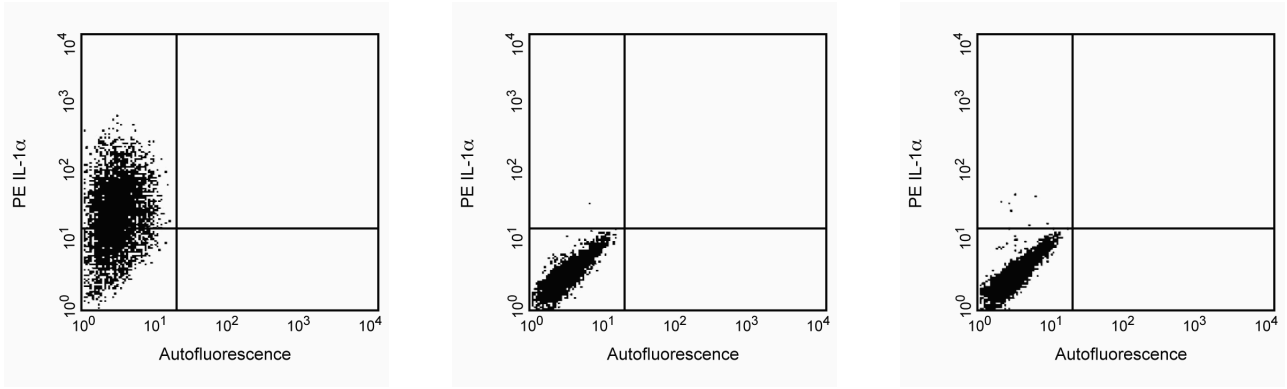
PE Hamster Anti-Mouse IL-1α

Product Information

Material Number:	**559810**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	ALF-161
Immunogen:	Mouse IL-1α recombinant protein
Isotype:	Armenian Hamster IgG1, λ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

This antibody recognizes the precursor, secreted and membrane-associated forms of mouse interleukin-1α (IL-1α) protein. No cross-reactivity was detected with mouse IL-1β. This antibody does not recognize human IL-1α or IL-1β. The cross-reactivity of this antibody with IL-1α from other species has not been tested. The immunogen used to generate this ALF-161 hybridoma was purified, recombinant mouse IL-1α protein. This is a neutralizing antibody.



Expression of IL-1α by activated mouse peritoneal macrophages. *Thioglycolate-elicited BALB/c mouse peritoneal macrophages were primed for 2 hour with rmIFN-γ (10 µg/ml, Cat. No. 554587) and stimulated overnight with LPS (1 µg/ml, Sigma Cat. No. L-8274) in the presence of BD GolgiPlug™ (containing Brefeldin A, Cat. No. 555029). The activated cells were harvested, fixed, permeabilized and stained with PE-conjugated anti-mouse IL-1a antibody (PE-ALF-161, Cat. No. 559810; left panel) following BD Biosciences Pharmingen's intracellular staining protocol. To demonstrate specificity of staining, the binding of PE- ALF-161 antibody was blocked by preincubation of the conjugated antibody with recombinant mouse IL-1α (0.25 µg, Cat. No. 551778; middle panel) or by preincubation of the fixed/ permeabilized cells with unlabelled ALF-161 antibody (5.0 µg; Cat. No. 550604; right panel) prior to staining with the PE- ALF-161 antibody. The quadrant markers for the bivariate dot plots were set based on the autofluorescence controls and verified using the recombinant cytokine blocking and unlabelled 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 Cytometry Analysis: The PE-conjugated ALF-161 antibody can be used for multicolor immunofluorescent staining and flow cytometric analyses to identify and enumerate IL-1α-producing cells within mixed cell populations (see figure). 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 ALF-161 antibody with its cognate ligand (e.g., recombinant mouse IL-1α) prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabelled ALF-161 antibody (Cat. No. 550604) prior to staining. The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable Armenian hamster IgG isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse or human cells is PE-G235-2356 (Cat. No. 554711); use at comparable concentrations to antibody of interest (e.g., ≤ 0.25 µg mAb/1 million cells).

ELISA: The ALF-161 antibody is useful as a capture antibody in a sandwich ELISA. Cat. No. 550604 is recommended for this application.

Neutralization: The ALF-161 antibody is useful for neutralization of mouse IL-1α bioactivity.

Western Blot: The purified ALF-161 antibody has been found useful for Western blotting. Please note that this application is not routinely tested at BD Biosciences Pharmingen.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554711	PE Hamster IgG1, λ1 Isotype Control	0.1 mg	G235-2356
554587	Recombinant Mouse IFN-γ Protein	10 µg	(none)
550604	Purified Hamster Anti-Mouse IL-1α	0.5 mg	ALF-161
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554654	Mick-3 Cytokine Positive Control Cells	5x10^6 cells	(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

Chang MJ, Modzelewski RA, Russell DM, Johnson CS. Interleukin 1 alpha and gamma-interferon induction of nitric oxide production from murine tumor-derived endothelial cells. *Cancer Res.* 1996; 56(4):886-891.(Biology)

Fuhlbrigge RC, Sheehan KC, Schreiber RD, Chaplin DD, Unanue ER. Monoclonal antibodies to murine IL-1 alpha. Production, characterization, and inhibition of membrane-associated IL-1 activity. *J Immunol.* 1988; 141(8):2643-2650.(Clone-specific: Neutralization)

Kitamura T, Takaku F, Miyajima A. IL-1 up-regulates the expression of cytokine receptors on a factor-dependent human hemopoietic cell line, TF-1. *Int Immunol.* 1991; 3(6):571-577.(Clone-specific: Neutralization)

Kitamura T, Tange T, Terasawa T, et al. Establishment and characterization of a unique human cell line that proliferates dependently on GM-CSF, IL-3, or erythropoietin. *J Cell Physiol.* 1989; 140(2):323-334.(Clone-specific: 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)