Technical Data Sheet

# PE Rat Anti-Human IL-3

## Product Information

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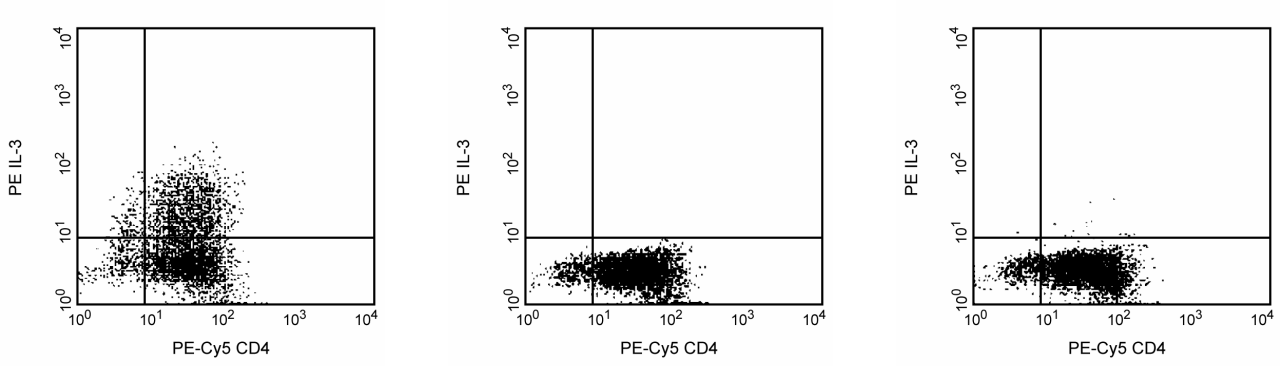
|---|---|
| **Material Number:** | **554676** |
| **Size:** | 0.1 mg |
| **Concentration:** | 0.2 mg/ml |
| **Clone:** | BVD3-1F9 |
| **Immunogen:** | Recombinant Human IL-3 |
| **Isotype:** | Rat IgG1 |
| **Reactivity:** | QC Testing: Human |
| **Storage Buffer:** | Aqueous buffered solution containing ≤0.09% sodium azide. |

## Description

The BVD3-1F9 antibody reacts with human interleukin-3 (IL-3). The immunogen used to generate the BVD3-1F9 hybridoma was recombinant human IL-3. This is a weakly neutralizing antibody.




***Expression of IL-3 by stimulated human CD4 cells.*** Isolated human CD4+ cells were stimulated with immobilized anti-human CD3 mouse antibody (UCHT1, Cat. No. 555329), soluble anti-human CD28 mouse antibody (CD28.2, Cat. No. 555725), recombinant human IL-2 (10 ng/ml final concentration; Cat. No. 554603) and recombinant human IL- 4 (20 ng/ml final concentration; Cat. No. 554605) for 2 days. The cells were subsequently cultured in medium containing recombinant human IL-2 and recombinant human IL- 4 for 3 days. Finally, the cells were harvested and re-stimulated for 6 hours with PMA (Sigma, Cat. #P-8139) and calcium ionophore A23187 (1 µg/ml final concentration; Sigma, Cat. #C-9275) in the presence of Golgi-Stop™ (2 µM final concentration; Cat. #554724). The cells were harvested, stained with Pe-Cy5 anti- CD4 (RPA-T4, Cat. No. 555348), fixed, permeabilized, and subsequently stained with 0.12 µg of PE-rat anti-human IL-3 antibody (PE-BVD3-1F9, Cat. No. 554676) (see Left panel). To demonstrate specificity of staining, the binding by PE- BVD3-1F9 antibody was blocked by each of the following: 1) preincubation of the fluorochrome-conjugated antibody with 0.5µg recombinant human IL-3 (see Center panel) and by 2) preincubation of the fixed/permeabilized cells with unlabeled BVD3-1F9 antibody (5 µg; Cat. No. 554673; see Right panel) prior to staining with the PE-BVD3- 1F9 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 by gel filtration chromatography.

Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

## Application Notes

**Application**

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| Intracellular staining (flow cytometry) | Routinely Tested |

**Recommended Assay Procedure:**

**Immunofluorescent Staining and Flow Cytometric Analysis:** The BVD3-1F9 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-3 producing cells within mixed cell populations. The PE-conjugated BVD3-1F9 antibody (Cat. No. 554676) is especially suitable for these studies. For optimal immunofluorescent staining and flow cytometric analysis, this anti-cytokine

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antibody should be titrated ( ≤ 0.5 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 suitable rat IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is R3-34 (Cat. No. 554685); use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg mAb/1 million cells). A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the conjugated BVD3-1F9 antibody with ligand (e.g., recombinant human IL-3; Cat No. 554604) prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabeled BVD3-1F9 antibody prior to staining. The staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

**ELISA**: The biotinylated BVD3-1F9 antibody (Cat. No. 554674) is useful as a detection antibody for a sandwich ELISA for measuring human IL-3 protein levels. Biotinylated BVD3-1F9 antibody can be paired with the purified BVD8-3G11 antibody (Cat. No. 554672) as the capture antibody, with recombinant human IL-3 protein (Cat. No. 554604) as the standard.

## Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|---|---|---|---|
| 554604 | Recombinant Human IL-3 | 10 µg | (none) |
| 554685 | PE Rat IgG1, κ Isotype Control | 0.1 mg | R3-34 |
| 555329 | Purified NA/LE Mouse Anti-Human CD3 | 0.5 mg | UCHT1 |
| 555725 | Purified NA/LE Mouse Anti-Human CD28 | 0.5 mg | CD28.2 |
| 554603 | Recombinant Human IL-2 | 5 µg | (none) |
| 554605 | Recombinant Human IL-4 | 5 µg | (none) |
| 554724 | Protein Transport Inhibitor (Containing Monensin) | 0.7 ml | (none) |
| 555348 | PE-Cy™5 Mouse Anti-Human CD4 | 100 tests | RPA-T4 |
| 554715 | BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop) | 250 tests | (none) |
| 554653 | Mick-2 Cytokine Positive Control Cells | NA | (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. 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, 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)

Abrams JS, Silver J, Van Dyke R, Gleich G. Eosinophil-active cytokines in human disease: development and use of monoclonal antibodies to IL-3, IL-5, GMCSF. In: Kay A and Gleich G, ed. *Eosinophils in Allergy and Inflammation.* 1994:133-157.(Clone-specific: ELISA)

Kaushansky K, Shoemaker SG, Broudy VC. Structure-function relationships of interleukin-3. An analysis based on the function and binding characteristics of a series of interspecies chimera of gibbon and murine interleukin-3. *J Clin Invest.* 1992; 90(5):1879-1888.(Clone-specific)

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)