## **Technical Data Sheet**

# PE Rat Anti-Human IL-12 p70

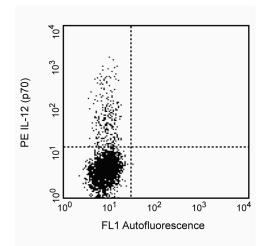
#### **Product Information**

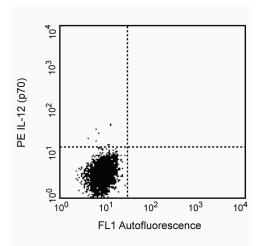
559325 **Material Number:** 100 tests 20 µl Vol. per Test: 20C2 Clone: Rat IgG1, ĸ Isotype: Reactivity: QC Testing: Human

Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

## Description

The 20C2 antibody reacts with the biologically active form of human IL-12 p70 heterodimer, but not with the p40 monomer or homodimer.





Specific control for intracellular staining of human IL-12 p70-producing cells. Pharmingen's HiCK-3 positive control cells (Cat. No. 555063) were intracellulary stained using Pharmingen's intracellular staining protocol. The panels show intracellular staining with PE-conjugated antihuman IL-12 p70 (0.06 μg) and the pre-blocking of that staining with purified anti-human IL-12 p70 (5.0 µg, see right panel).

## 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**

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Routinely Tested Intracellular staining (flow cytometry)

## **Recommended Assay Procedure:**

Immunofluorescent Staining and Flow Cytometric Analysis: The 20C2 antibody is useful for immunofluorescent staining and flow cytometric analysis of mixed cell populations to identify and enumerate IL-12 p70 producing cells. This 100 Test Size formulation of the PE-conjugated 20C2 antibody has been pre-titrated to assure effective intracellular detection of human IL-12 p70 using 20 μl/1 x 10<sup>6</sup> cells.

A suitable rat IgG1 isotype control for assessing the level of fluorochrome associate background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is also available in a 100 Test Size formulation PE-R3-34 immunoglobulin (Cat. No. 559318). A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the fluorochrome-conjugated 20C2 antibody with excess ligand (e.g., recombinant human IL-12 p70, Cat. No. 554613) prior to staining, or 2) pre-block the fixed/permeabilized cells

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with unlabeled 20C2 antibody (Cat. No. 557018) prior to staining. The intracellular staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

Important Note: This pre-titered antibody solution does not contain a cell permeabilization agent. It is necessary to include a cell permeabilization agent when using the pre-titered antibody solution to stain fixed and permeabilized cells. Perm/Wash<sup>TM</sup> Buffer (Cat. No. 554723) contains the permeabilization agent saponin and is useful for this purpose.

## **Suggested Companion Products**

Catalog Number	Name	Size	Clone
559318	PE Rat IgG1, κ Isotype Control	100 tests	R3-34
555063	HiCK-3 Human Cytokine Positive Control Cells	1.0 ml	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)

#### **Product Notices**

- This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10<sup>6</sup> cells in a 100-µl experimental sample (a test).
- 2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- 4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

#### References

Gately MK, Chizzonite R, Presky DH. Measurement of Human and Mouse Interleukin-12. In: Cooligan J, Kruisbeek A, Margulies D, Shevach E, Storber W, ed. Current Protocols in Immunology. New York: John Wiley and Sons; 1995:6-16. (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)

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