Technical Data Sheet

PE Mouse Anti-Stat1 (pY701)

Product Information

Material Number: 562069 Size: 250 tests 5 µl Vol. per Test: Clone:

Immunogen: Phosphorylated Human Stat1 Peptide

Isotype: Mouse IgG2a Reactivity: QC Testing: Human

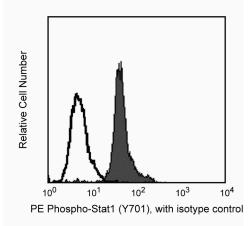
Tested in Development: Mouse

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

Description

Stat (Signal transducer and activators of transcription) proteins are critical mediators of the biologic activity of cytokines, including interleukins, interferons, erythropoietin, and growth factors. Ligand-receptor interaction leads to activation of constitutively associated JAK family kinases and subsequent recruitment/activation of Stat proteins by tyrosine phosphorylation. Active Stat proteins then move to the nucleus to promote transcription of cytokine-inducible genes. Seven Stat proteins have been cloned, each of which is differentially expressed and/or activated in a cytokine-specific and cell type-specific manner. Stat1 and Stat2 are components of the ISGF3 (Interferon-Stimulated Gene Factor 3) complex, which is the primary transcription activator induced by the binding of the interferon to a specific cell-surface receptor. Stat1 has two alternatively spliced isoforms, 91-kDa Stat1α and 84-kDa Stat1β; Stat1α has 38 additional C-terminal amino acids. In response to the binding of IFNα, IFNγ, EGF, PDGF, or CSF-1 to their respective receptors, the Stat1 subunits become tyrosine-phosphorylated at Y701, and the complex is translocated to the nucleus. This results in the formation of an active complex that includes the DNA-binding p48 subunit. This complex is responsible for modulating the transcription of the interferon-stimulated genes (ISGs).

The 4a monoclonal antibody recognizes the phosphorylated Y701 in Stat1α and Stat1β.



Flow cytometric analysis of phospho-Stat1 (pY701). Human histiocytic lymphoma cells (U937) were either lelft unstimulated (unshaded)or stimulated (shaded) with 1000 U/ml BD Pharmingen™ Recombinant Human IFNy (cat. No. 554617) for 15 minutes at 37°C. Cells were fixed in 2% paraformaldehyde/PBS (10 minutes at 37°C) and then permeabilized in BD Phosflow™ Perm Buffer III (30 minutes on ice or overnight at -20°C). Cells were then washed twice in BD Pharmingen™ Stain Buffer (Cat. No. 554656) and stained with PE-conjugated phospho-Stat1 (pY701) antibody. The cells were analyzed on a BD FACSCalibur $^{\text{TM}}$

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:

For more information on intracellular flow cytometry and appropriate buffer selection please visit http://www.bdbiosciences.com/support/resources/phosflow/index.jsp and http://www.cytobank.org/facselect/

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Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|----------------|-------------------------|--------|--------|
| 554655 | Fixation Buffer | 100 ml | (none) |
| 558050 | Perm Buffer III | 125 ml | (none) |
| 554656 | Stain Buffer (FBS) | 500 ml | (none) |
| 554617 | Recombinant Human IFN-γ | 50 ug | (none) |

Product Notices

- 1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100- μ l 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. 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.
- 5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- 6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.

References

Bromberg J, Darnell JE. The role of STATs in transcriptional control and their impact on cellular function. *Oncogene*. 2000; 19(21):2468-2473. (Biology) Darnell JE Jr. STATs and gene regulation. *Science*. 1997; 277(5332):1630-1635. (Biology)

Fu XY, Zhang JJ. Transcription factor p91 interacts with the epidermal growth factor receptor and mediates activation of the c-fos gene promoter. *Cell.* 1993; 74(6):1135-1145. (Biology)

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