

## Technical Data Sheet

## Alexa Fluor® 647 Mouse Anti-Stat4 (pY693)

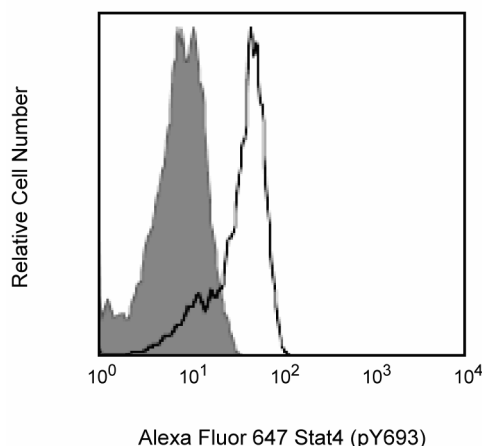
## Product Information

<b>Material Number:</b>	558137
<b>Alternate Name:</b>	Signal transducer and activator of transcription 4; SLEB11
<b>Size:</b>	50 Tests
<b>Vol. per Test:</b>	20 µl
<b>Clone:</b>	38/p-Stat4
<b>Immunogen:</b>	Phosphorylated Human Stat4 (pY693)
<b>Isotype:</b>	Mouse IgG2b, κ
<b>Reactivity:</b>	QC Testing: Human Tested in Development: Mouse
<b>Storage Buffer:</b>	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

## Description

The Stat proteins function both as cytoplasmic signal transducers and as activators of transcription. Seven mammalian Stat proteins have been identified: Stat1-4, Stat5a, 5b, and Stat6. Stat4 has been shown to play an important role in development of T helper cells, specifically the Th1 subset. Stat4 is activated by IL-12 and by type 1 interferons. Knockout mice supported the role that Stat4 plays in IL-12 signaling because lymphocytes from Stat 4<sup>-/-</sup> mice could neither differentiate into Th1 cells nor produce IFN-γ in response to treatment with IL-12. IFN-γ plays an important role in host defense. A key component in the activation of Stat4 is the phosphorylation on tyrosine and serine residues in response to IL-12 stimulation. IL-12 stimulation leads to the phosphorylation of Stat4 on tyrosine 693 and serine 721. Transcriptional activity of Stat4 has been shown to be significantly reduced when residues Y693 and S721 are mutated.

Clone 38/p-Stat4 has been confirmed to recognize mouse phospho-Stat4 with western blot application using Purified Mouse Anti-Stat4 (pY693), Cat. Nos. 612739 & 612738.



**Analysis of Stat4 (pY693) in activated human peripheral blood lymphocytes.** Human whole blood was either stimulated with 40,000 U/ml of IFN-α for 15 minutes at 37°C (open histogram) or unstimulated (shaded histogram). The cells were lysed and fixed with 1X BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049) for 10-15 minutes at 37°C, then permeabilized using BD Phosflow™ Perm Buffer III, (Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 647 anti-Stat4 (pY693). For data analysis, lymphocytes were selected by scatter profile. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.

## 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® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

## Application Notes

## Application

Intracellular staining (flow cytometry)	Routinely Tested
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## Suggested Companion Products

Catalog Number	Name	Size	Clone
558049	Lyse/Fix Buffer 5X	250 mL	(none)
558050	Perm Buffer III	125 mL	(none)

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554656	Stain Buffer (FBS)	500 mL	(none)
554655	Fixation Buffer	100 mL	(none)

## Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100- $\mu$ L experimental sample (a test).
2. Please refer to [www.bdbiosciences.com/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/protocols) for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
7. 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.
8. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
9. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

## References

Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW. Signaling through the JAK/STAT pathway, recent advances and future challenges. *Gene*. 2002; 285:1-24. (Biology)

Visconti R, Gadina M, Chiariello M, Chen EH, Stancato LF, Gutkind JS, O'Shea J. Importance of the MKK6/p38 pathway for interleukin-12-induced STAT4 serine phosphorylation and transcriptional activity. *Blood*. 2000; 96:1844-1852. (Biology)

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