

Phospho-Stat3 (Tyr705) (D3A7) Rabbit mAb (Alexa Fluor® 647 Conjugate)

✓ 500 µl
(50 tests)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

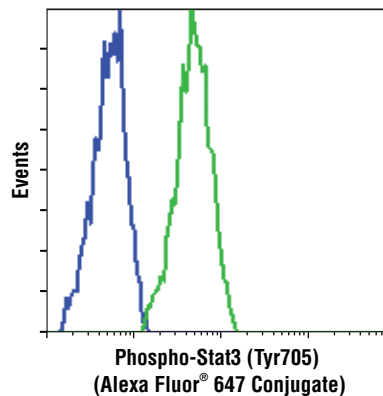
Applications	Species Cross-Reactivity*	Isotype
F Endogenous	H, M, R	Rabbit IgG

Description: This Cell Signaling Technology antibody is conjugated to Alexa Fluor® 647 fluorescent dye and tested in-house for direct flow cytometric analysis of human cells. The unconjugated Phospho-Stat3 (Tyr705) (D3A7) Rabbit mAb #9145 reacts with Phospho-Stat3 (Tyr705) from human, mouse and rat. CST expects that Phospho-Stat3 (Tyr705) (D3A7) Rabbit mAb (Alexa Fluor® 647 Conjugate) will also recognize Phospho-Stat3 in these species.

Background: The Stat3 transcription factor is an important signaling molecule for many cytokines and growth-factor receptors (1) and is required for murine fetal development (2). Stat3 is constitutively activated in a number of human tumors (3,4) and possesses oncogenic potential (5) and anti-apoptotic activities (3). Stat3 is activated by phosphorylation at Tyr705, which induces dimerization, nuclear translocation and DNA binding (6,7). Transcriptional activation seems to be regulated by phosphorylation at Ser727 through the MAPK or mTOR pathways (8,9). Stat3 isoform expression appears to reflect biological function as the relative expression levels of Stat3α (86 kDa) and Stat3β (79 kDa) depend on cell type, ligand exposure or cell maturation stage (10). It is notable that Stat3β lacks the serine phosphorylation site within the carboxy-terminal transcriptional activation domain (8).

Specificity/Sensitivity: Phospho-Stat3 (Tyr705) (D3A7) Rabbit mAb (Alexa Fluor® 647 Conjugate) detects endogenous levels of Stat3 only when phosphorylated at Tyr705. This antibody does not cross-react with phospho-EGFR or with corresponding phospho-tyrosines of other Stat proteins.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Tyr705 of mouse Stat3. The antibody was conjugated to Alexa Fluor® 647 under optimal conditions with an F/P ratio of 2-6. The Alexa Fluor® 647 dye is maximally excited by red light (e.g. 633 nm He-Ne laser). Antibody conjugates of the Alexa Fluor® 647 dye produce bright far-red-fluorescence emission with a peak at 665 nm.



Flow cytometric analysis of Jurkat cells, untreated (blue) or IFN-α treated (green), using Phospho-Stat3 (Tyr705) (D3A7) Rabbit mAb (Alexa Fluor® 647 Conjugate).

Background References:

- (1) Heim, M.H. (1999) *J. Recept. Signal Transduct. Res.* 19, 75-120.
- (2) Takeda, K. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 3801-3804.
- (3) Catlett-Falcone, R. et al. (1999) *Immunity* 10, 105-115.
- (4) Garcia, R. and Jove, R. (1998) *J. Biomed. Sci.* 5, 79-85.
- (5) Bromberg, J.F. et al. (1999) *Cell* 98, 295-303.
- (6) Darnell Jr., J.E. et al. (1994) *Science* 264, 1415-1421.
- (7) Ihle, J.N. (1995) *Nature* 377, 591-594.
- (8) Wen, Z. et al. (1995) *Cell* 82, 241-250.
- (9) Yokogami, K. et al. (2000) *Curr. Biol.* 10, 47-50.
- (10) Biethahn, S. et al. (1999) *Exp. Hematol.* 27, 885-894.

Entrez-Gene ID #6774
Swiss-Prot Acc. #P40763

Storage: Supplied in PBS (pH 7.2), less than 0.1% sodium azide, 2 mg/ml BSA. Store at 4°C. *Protect from light. Do not freeze.*

***Species cross-reactivity other than human is determined by Western blot using the unconjugated antibody.**

Recommended Antibody Dilutions:
Flow Cytometry 1:10

Directions for Use: Add 10 µl of the conjugated antibody to 500,000 cells in 90 µl PBS/0.5% BSA. See protocol for more details.

Companion Products:

Phospho-Stat3 (Tyr705) Antibody #9131

Phospho-Stat3 (Tyr705) (3E2) Mouse mAb #9138

Stat3 (124H6) Mouse mAb #9139

Phospho-Stat3 (Tyr705) (D3A7) Rabbit mAb (Alexa Fluor® 488 Conjugate) #4323

Phospho-Stat3 (Tyr705) (D3A7) Rabbit mAb #9145

Rabbit (DA1E) mAb IgG Isotype Control (Alexa Fluor® 647 Conjugate) #2985

The Alexa Fluor® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc., for research use only, except for use in combination with DNA microarrays. The Alexa Fluor® dyes (except for Alexa Fluor® 430 dye) are covered by pending and issued patents.

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Flow Cytometry Protocol for Intracellular Staining Using Conjugated Primary Antibodies

A Solutions and Reagents

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 ml distilled water (dH_2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100ml 1X PBS. Store at 4°C

B Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
3. Fix for 10 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

C Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at -20°C in 90% methanol.

D Staining Using Conjugated Primary Antibodies

NOTE: Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

1. Aliquot 5×10^5 cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation.
3. Resuspend cells in 90 μl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add 10 μl of conjugated antibody to the assay tubes.
6. Incubate for 30-60 minutes, in the dark at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.