

# Anti-phospho-STAT3 (Tyr705), clone EP2147Y, Rabbit Monoclonal

Monoclonal Antibody

Cat. # 04-1059

Lot # NRG1635952

pack size: 100 µL

Store at -20°C

FOR RESEARCH USE ONLY



## Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IH(P), IC, IP	H, M	IgG	Phosphorylated Tyr705	Rb	~84 or 76 kDa	NP_644805.1

### Background

STAT proteins (Signal Transduction and Activators of Transcription) are latent cytoplasmic transcription factors that have the dual function of signal transduction and activation of transcription. STATs are activated by tyrosine phosphorylation in response to different ligands, after which they translocate to the cell nucleus. The N-terminal region is highly homologous among the STAT proteins and surrounds a completely conserved arginine residue. STATs are a part of the JAK-STAT signaling pathway – a major pathway of the immune system. All cytokines transduce critical signals through this pathway. STAT3 has been shown to be activated by IFN alpha but not IFN beta. The transcription factors associated with STAT3 are cJun and cyclic AMP responsive enhancer binding protein (CREB). Deletion of the STAT3 gene in knock out mice was lethal at the early embryonic stage.

### Presentation

Rabbit Monoclonal in buffer containing 50 mM Tris-Glycine (pH 7.4), 0.15 M NaCl containing 40% Glycerol, 0.01% sodium azide and 0.05% BSA.

### Specificity

This antibody recognizes STAT3 phosphorylated Tyr705.

### Species Cross-reactivity

Human and mouse.

### Immunogen

Synthetic peptide corresponding to residues surrounding phosphorylated Tyr705 of human STAT3.

### Molecular Weight

Observed MW: STAT3α (~84 kDa) and STAT3β (~76 kDa), depending on cell type.

### Method of Purification

Unpurified

### Storage and Handling

Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon receipt and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.

Note: Variability in freezer temperatures below -20°C may cause glycerol containing solutions to become frozen during storage.

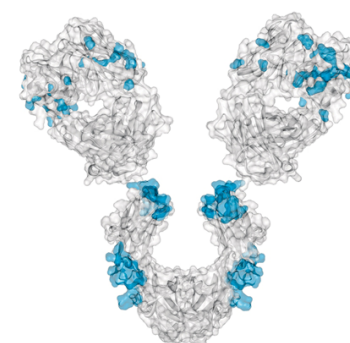
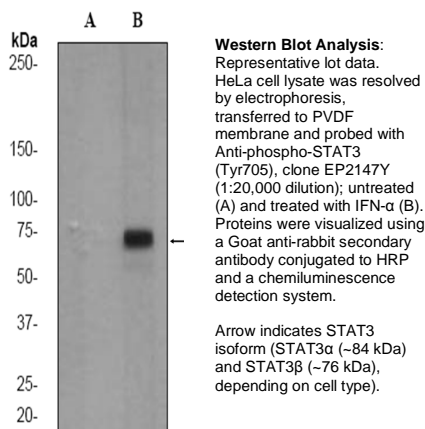
### Control

HeLa cell lysate

### Quality Control Testing

Evaluated by Western Blot on untreated and IFN-α-treated HeLa cell lysates.

**Western Blot Analysis:** A 1:10,000-20,000 dilution of this antibody was used to detect STAT3 in IFNα-treated HeLa cell lysate.



### References

- Heim, M., et al. (1999). *Signal Transduct. Res.* 19: 75–120.
- Kisseleva, T. et al. (2002). *Gene* 285:1–24.]
- Bromberg, J.F. et al. (1999). *Cell* 98:295–303.
- Wen, Z. et al. (1995). *Cell* 82: 241–250.

### Additional Research Applications

**Immunohistochemistry Analysis:** A 1:100-250 dilution from a previous lot detected STAT3 in human colon adenocarcinoma tissue.

**Immunocytochemistry Analysis:** A 1:100-250 dilution from a previous lot detected STAT3 in HeLa cells.

**Immunoprecipitation:** A 1:40 dilution from a previous lot was used in IP.

**APPLICATION LEGEND:** WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence IH Immunohistochemistry (Tissue) IH(P) Immunohistochemistry (Paraffin) FC Flow Cytometry

**SPECIES LEGEND:** H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

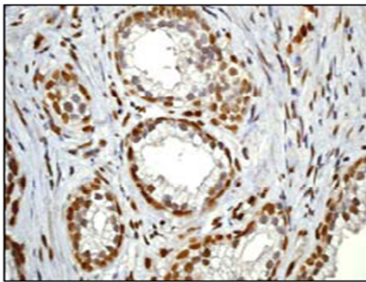
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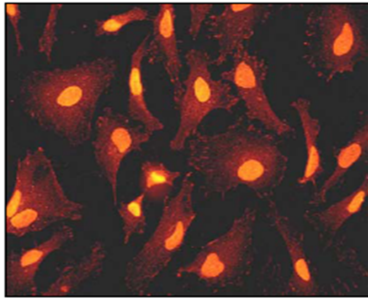
**Additional Research Applications**



Immunohistochemistry (Paraffin) Analysis:

Representative lot data.

Immunohistochemical staining of paraffin-embedded human colon adenocarcinoma using Anti-phospho-STAT3 (Tyr705), clone EP2147Y.



Immunofluorescent IC Analysis:

Representative lot data.

Immunofluorescent staining of HeLa cells using Anti-phospho-STAT3 (Tyr705), clone EP2147Y.

**PROTOCOL**

**Western Blot**

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on cell lysate and transfer the proteins to a PVDF membrane. Wash the PVDF membrane twice with water.
2. Block the blotted PVDF membrane in freshly prepared 5% BSA or milk with 0.05% Tween<sup>®</sup>-20 for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with the recommended dilution of anti-phospho-STAT3 (Tyr705), clone EP2147Y diluted in freshly prepared 5% BSA or milk for 1 hour at room temperature or overnight with agitation at 2-8°C.
4. Wash the PVDF 3 times with TBST.
5. Incubate the PVDF in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348 1:1000 dilution was used) in 5% milk for 1 hour with agitation at room temperature.
6. Wash the PVDF 3-5 times with TBST.
7. Use Spray and Glow Catalog # 17-373 to visualize results. Use as directed.

**Produced in collaboration with Epitomics™**

**RELATED PRODUCTS (specific)**

cat #	description
12-348	■ Goat Anti-Rabbit IgG, HRP-conjugate

**RELATED PRODUCTS (non-specific)**

cat #	description
WBAVDBASE	■ SNAP i.d. Protein Detection System
WBAVDABTR	■ SNAP i.d. Antibody Collection Tray
WBAVDR0LL	■ SNAP i.d. Blot Roller
WBAVDBH03	■ SNAP i.d. Triple Well Blot Holder
WBAVDBH01	■ SNAP i.d. Single Well Blot Holder
WBAVDBH02	■ SNAP i.d. Double Well Blot Holder
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 μm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 μm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 μm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

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