

MONOCLONAL ANTIBODY

# Anti-Phospho-STAT3 (Tyr708) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D128-3	PS3/1	Mouse IgG1	100 $\mu$ L	1 mg/mL

**BACKGROUND:** STAT (Signal Transducers and Activators of Transcription) proteins play important roles in development, cell differentiation and cell cycle control. STAT3 is an ~85 kDa protein involved in the signaling pathways of many cytokines and growth factors, including G-CSF and IL-6, where it functions as a negative regulator of transcription. STAT3 is also constitutively activated in a number of human tumors and it possesses anti-apoptotic activity and oncogenic potential. STAT3 may also regulate apoptosis by inhibiting NF- $\kappa$ B. Activation of STAT3 by tyrosine phosphorylation results in dimerization, nuclear translocation and DNA binding.

**SOURCE:** This antibody was purified from hybridoma (clone PS3/1) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Balb/c mouse splenocyte immunized with the 12 amino acids peptide CVTQPpYLKTKFI (703~714 aa) which contained the phosphorylated tyrosine 708 of zebrafish STAT3.

**FORMULATION:** 100  $\mu$ g IgG in 100  $\mu$ L volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

**STORAGE:** This antibody solution is stable for one year from the date of purchase when stored at  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody reacts with human and mouse STAT3 phosphorylated at Tyr705, and zebrafish STAT3 phosphorylated at Tyr708.

**APPLICATIONS:**

- Western blotting; 1  $\mu$ g/mL
- Immunoprecipitation; Not tested
- Immunohistochemistry; Not tested
- Immunocytochemistry; Not tested
- Flow cytometry; Not tested

Detailed procedure is provided in the following PROTOCOL.

**INTENDED USE:**

For Research Use Only. Not for use in diagnostic procedures.

**SPECIES CROSS REACTIVITY:**

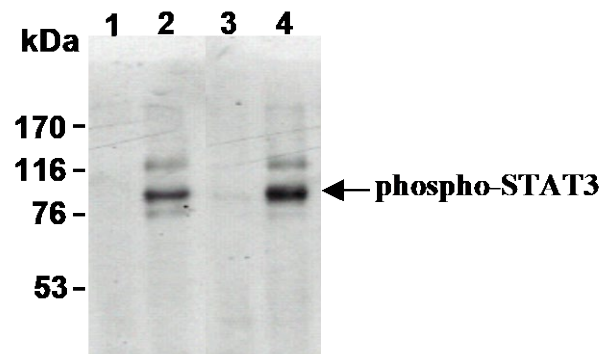
Species	Human	Mouse	Rat	Zebrafish
Cells	A431 stimulated with EGF	Transfectant	Not tested	Transfectant
Reactivity on WB	+	+		+

**REFERENCE:**

- 1) Yamashita, S., *et al.*, *Dev. Cell* **2**, 363-375 (2002)

Clone PS3/1 is used in this reference.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.



**Western blot analysis of STAT3 phosphorylation in zebrafish STAT3 transfected 293T (1), zebrafish STAT3 and mouse JAK1 co-transfected 293T (2), mouse STAT3 transfected 293T (3) and mouse STAT3 and mouse JAK1 co-transfected 293T (4) using D128-3.**

**PROTOCOL:**

**SDS-PAGE & Western Blotting**

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at  $4^{\circ}\text{C}$  with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at  $4^{\circ}\text{C}$  and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis

buffer to make 8 mg/mL solution.

- 3) Mix the sample with equal volume of Laemmli's sample buffer.
- 4) Boil the samples for 2 minutes and centrifuge. Load 10  $\mu$ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hour in a semi-dry transfer system. (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
- 6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
- 9) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 10) Wash the membrane with PBS-T (5 minutes x 6 times).
- 11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Transfectant)

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