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

MBL
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MONOCLONAL ANTIBODY

Anti-Phospho-STAT3 (Tyr708) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D128-3	PS3/1	Mouse IgG1	100 µL	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-κB. 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 µg IgG in 100 µL 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°C.

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

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

Detailed procedure is provided in the following PROTOCOL.

INTENDED USE:

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SPECIES CROSS REACTIVITY:

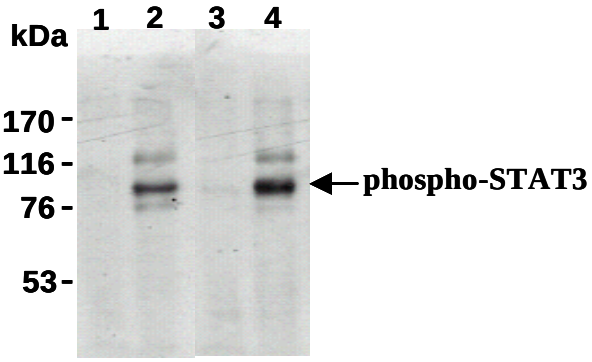
Species	Human	Mouse	Rat	Zebrafish
Cells	A431 stimulated with EGF	Transfectant	Not tested	Transfectant
Reactivity on WB	+	+		+

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°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°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 μL 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^2 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)