

## Product datasheet for **AM26475AF-N**

### STAT3 pTyr705 (703-714) Mouse Monoclonal Antibody [Clone ID: PS3/1]

#### Product data:

Product Type:	Primary Antibodies
Clone Name:	PS3/1
Applications:	WB
Recommended Dilution:	Western blot: 1 µg/ml. For details see protocol below.
Reactivity:	Human, Mouse, Zebrafish
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	12 amino acids peptide (a.a. 703~714) which contained the phosphorylated Tyr708 of zebrafish STAT3
Specificity:	This antibody reacts with human and mouse STAT3 phosphorylated at Tyr705, and zebrafish STAT3 phosphorylated at Tyr708.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein-A agarose
Conjugation:	Unconjugated
Storage:	Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	signal transducer and activator of transcription 3
Database Link:	<a href="#">Entrez Gene 6774 Human P40763</a>



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**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 GCSF 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.

**Synonyms:** STAT-3, Acute-phase response factor, APRF

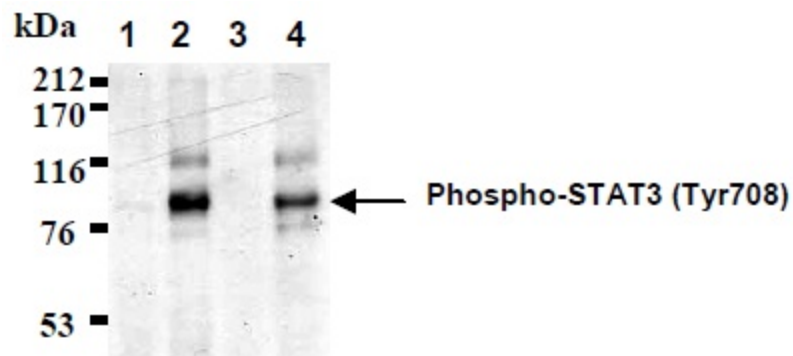
**Note:** This product was originally produced by MBL International.

**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 oC 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 oC 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 oC.
- 7) Incubate the membrane with the anti-phospho-zebrafish STAT3 (Tyr708) monoclonal antibody (1  $\mu$ g/mL) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 8) Wash the membrane with PBS (5 minutes x 6 times).
- 9) Incubate the membrane with the 1:10000 POD-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 10) Wash the membrane with PBS (5 minutes x 6 times).
- 11) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagents for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
- 12) Expose to X-ray film in a dark room for 5 minutes. Develop the film as usual. The conditions for exposure and development may vary.

## Product images:



Western blot analysis of STAT3 phosphorylation using AM26475AF-N in 293T cells transfected with zebrafish STAT3 (1), 293T cells co-transfected with zebrafish STAT3 and mouse JAK1 (2), 293T cells transfected with mouse STAT3 (3), 293T cells cotransfected with mouse STAT3 and mouse JAK1 (4).