3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Anti-phospho-ATF-2 (pThr^{71})
produced in rabbit, affinity isolated antibody

Catalog Number **A8853**

Product Description

Anti-phospho-ATF-2 (Activating Transcription Factor 2) (pThr^{71}) is produced in rabbit using as immunogen a synthetic phosphorylated peptide derived from the region of human ATF2 (Gene ID# 1386) that contains Thr^{71}. The sequence is conserved in mouse, chicken, frog, and rat. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated peptide.

The antibody detects human ATF-2. Mouse, rat, chicken and frog have not been tested but are expected to react (100% homology). It has been used in immunoblotting applications.

Activating transcription factor 2 (ATF-2) is a ubiquitously expressed member of the ATF/cAMP response element-binding protein family of basic region-leucine zipper proteins (bZIP), which play an important role in the cellular stress response and oncogenic transformation. ATF-2 is also believed to play a role in c-Jun-dependent cell cycle progression, cell survival and apoptosis. Other signaling enzymes involved in c-Jun/ATF-2 activation are the Src family kinases, which are downstream targets of mitogen-induced Ral activity.

Phosphorylation of threonines 69 and 71, mediated by JNK and p38, is essential for ATF-2 transcriptional activity. These sites appear to enhance the intrinsic histone acetyltransferase activity of ATF-2 and to regulate its degradation by the ubiquitin pathway. Threonine 71 can also be selectively phosphorylated by activation of the Ras →Raf →ERK1&2 pathways.

Reagent

Supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 1.0 mg/mL BSA (IgG and protease free) and 0.05% sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

One vial is sufficient for 10 immunoblots.

Immunoblotting: a recommended working concentration of 0.35-1.0 µg/mL is determined by using NIH3T3 cells.

Note: In order to obtain the best results in various techniques and preparations, we recommend determining optimal working concentration by titration.

Peptide Competition

1. NIH3T3 cells were left untreated (Lane 1) or treated with anisomycin (Lane 2-6) and cell lysates were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:

Lane 1and 4	no peptide
Lane 2	immunogen
Lane 3	non-phosphorylated peptide corresponding to the immunogen
Lane 4	a peptide containing generic phosphothreonine.

4. After preincubation membranes were incubated with 0.50 µg/mL ATF2 [pThr^{71}] antibody for two hours at room temperature in a 3% BSA-TBST buffer.
5. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase and signals were detected.

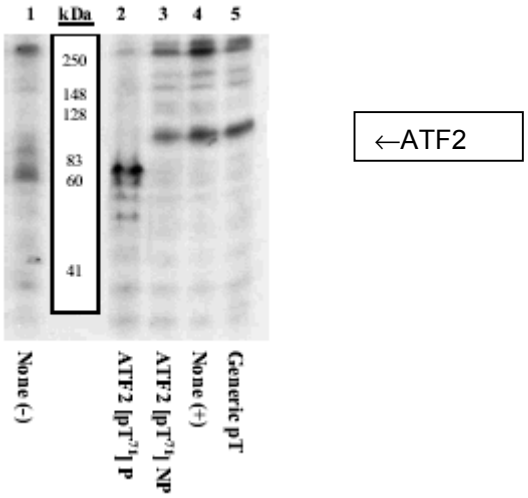

Figure 1 Peptide competition

The data in Figure 1 show that only the peptide corresponding to Anti-phospho-ATF2 (pThr^{71}) blocks the antibody signal, thereby demonstrating the specificity of the antibody.

References

1. Bhoumik, A., et al., An ATF2-derived peptide sensitizes melanomas to apoptosis and inhibits their growth and metastasis. *J Clin. Invest*., **110**, 643-650 (2002).
2. Ouwens, D.M., et al., Growth factors can activate ATF2 via a two-step mechanism: phosphorylation of Thr71 through the Ras-MEK-ERK pathway and of Thr69 through RalGDS-Srcp38. *EMBO J.,* **21**, 3782-379 (2002).
3. Waas, W.F., et al., The kinetic mechanism of the dual phosphorylation of the ATF2 transcription factor by p38 mitogen-activated protein (MAP) kinase. Implications for signal/response profiles of MAP kinase pathways. *J. Biol. Chem*., **276**, 5676-5684 (2001).
4. Miethe, J., et al., Crosstalk between Myc and activating transcription factor 2 (ATF2): Myc prolongs the half-life and induces phosphorylation of ATF2. *Oncogene*. **20**, 8116-8124 (2001).
5. Fuchs, S.Y., et al., Stability of the ATF2 transcription factor is regulated by phosphorylation and dephosphorylation. *J. Biol. Chem*., **275**, 12560-12564 (2000).

KAA,PHC 09/07-1