

Product Information

Anti-phospho-ATF-2 (pThr^{69,71}) antibody, Mouse monoclonal

clone ATF-22P, purified from hybridoma cell culture

Product Number **A4095**

Product Description

Anti-phospho-ATF-2 (pThr^{69,71}) antibody, Mouse monoclonal (mouse IgG1 isotype) is derived from the ATF-22P hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to the N-terminal amino acids 66-79 (pT^{69,71}) of human ATF2 conjugated to KLH. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Product Number ISO2. The antibody is purified from culture supernatant of hybridoma cells, grown in a bioreactor.

Monoclonal Anti-phospho-ATF2 (pT^{69,71}) reacts specifically with ATF2 phosphorylated at both threonine 69 and 71, and does not detect the monophosphorylated and the non-phosphorylated ATF2 molecule. The epitope recognized by the antibody resides in the N-terminal region of ATF2 (a.a. 66-79, pT^{69,71}). This epitope is identical in ATF2 of human, rat, mouse, chicken and frog. The antibody may be used in ELISA, immunoblotting (a triplet at 60-70 kDa) and immunocytochemistry. Cross-reactivity has been observed with human, rat and mouse ATF2.

Interaction of sequence-specific DNA-binding proteins with one or more regulatory elements controls the rate of transcriptional initiation from eukaryotic polymerase II promoters. These elements can be bound by a multiplicity of related proteins, thereby extending the repertoire of signals influencing the regulation of gene expression through individual regulatory sequences. The major regulators of the *c-jun* promoter are activating transcription factor 2 (ATF2, also called ATF-2 and CRE-BP1) and c-Jun. ATF2 (approx. 65 kDa) binds to both AP-1 and CRE DNA response elements, and is a member of the ATF/CREB family of leucine zipper proteins.^{1,2} The ATF/CREB family consists of a series of transcription factors that function through binding to the cAMP responsive element (CRE) palindromic octanucleotide TGACCTCA. Members of this gene family include CREB-1, CREB-2, ATF1, ATF2, ATF3 and ATF4. They share highly-related C-terminal leucine zipper and basic DNA binding domains (bZip motif), but are highly divergent in their N-terminal transactivating domains.

The transactivating potential of ATF2 is stimulated to a higher extent than that of c-Jun by a broad group of genotoxic agents causing DNA damage, and by other types of cellular stress such as inflammatory cytokines, short-wavelength UV, or alkylating compounds.³ ATF2 is known to interact with a variety of viral oncoproteins and cellular tumor suppressors, and is a target of the SAPK/JNK kinase signaling pathway.^{3,5} ATF2 heterodimerization with specific bZip proteins is an important determinant of the ubiquitination and proteasome-dependent degradation of ATF2, which determines how the magnitude and the duration of the cellular stress response are regulated.⁶ ATF2 contains two functional domains, an N-terminal transactivation domain and a C-terminal DNA-binding domain. The DNA-binding domain contains the basic leucine zipper (bZip) motif. The transactivation domain consists of two subdomains. The structure of an N-terminal half (N-subdomain) is well determined, while a C-terminal half (C-subdomain) has a highly flexible and disordered structure. The flexible C-subdomain contains two threonine residues (Thr69 and Thr71) that the stress-activated protein kinases (SAPK/JNK) phosphorylate. Following binding to a specific target protein, the C-subdomain undergoes a conformational change.⁷ Mutations of these sites reduce the ability of E1A and Rb to stimulate gene expression via ATF2.⁴ Antibodies reacting specifically with the phosphorylated ATF2 are useful tools in the study of the detailed mechanisms of transcription control in intracellular pathways, and its essential roles during developmental and pathological processes.

Reagents

Supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as a preservative.

Antibody Concentration: 0.5 -1.0 mg/ml.

Precautions and Disclaimer

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

Storage/Stability

For continuous use, store at 2-8°C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working concentration of 0.5-1 µg/ml is determined using a whole extract of cultured human acute T cell leukemia Jurkat, cells activated with anisomycin.

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

References

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