

Product Information

**Monoclonal Anti-phospho-ATF2 (pT^{69,71})
Peroxidase Conjugate**
Clone ATF-22P
Purified Mouse Immunoglobulin

Product Number **A 6228**

Product Description

Monoclonal Anti-phospho-ATF2 (pT^{69,71}), Peroxidase Conjugate is a lyophilized solution of a protein A purified fraction of monoclonal anti-phospho-ATF2 (pT^{69,71}), conjugated to horseradish peroxidase. monoclonal anti-phospho-ATF2 (pT^{69,71}) (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 antibody is purified from culture supernatant of hybridoma cells grown in a bioreactor.

Monoclonal Anti-phospho-ATF2 (pT^{69,71}), Peroxidase Conjugate reacts specifically with ATF2 phosphorylated at both Thr⁶⁹ and Thr⁷¹, and does not detect the corresponding monophosphorylated and the non-phosphorylated ATF2 molecule. The epitope recognized by the antibody resides in the N-terminal region of ATF2 (amino acids 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 (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 a 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 the N-terminal portion (N-subdomain) is well determined, while the C-terminal portion (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 for studying the detailed mechanisms of transcription control in intracellular pathways, and its essential roles during developmental and pathological processes.

Reagent

Monoclonal Anti-phospho-ATF2 (pT^{69,71}), Peroxidase Conjugate is supplied as a lyophilized powder. After reconstitution, the solution contains 0.01 M phosphate buffered saline, pH 7.4, 1% bovine serum albumin (BSA), and 0.01% merthiolate as a preservative.

Antibody concentration: 2-4 mg/ml.
Molar ratio Ab/E: 0.6-1.2
Enzyme activity: 150-500 U/ml

Storage/Stability

Store the lyophilized product at 2-8 °C. For extended storage after reconstitution, it is recommended to freeze in working aliquots. Repeated freezing and thawing is not recommended. 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.

Preparation Instructions

Reconstitute the vial with 0.2 ml of distilled water.

Product Profile

For immunoblotting, a working antibody concentration of 1-2 µg/ml is recommended using a whole extract of cultured human acute T cell leukemia Jurkat cells activated with anisomycin.

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

References

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