



Background: The transcription factor ATF-2 (also called CRE-BP1) binds to both AP-1 and CRE DNA response elements and is a member of the ATF/CREB family of leucine zipper proteins (1). ATF-2 interacts with a variety of viral oncoproteins and cellular tumor suppressors and is a target of the SAPK/JNK and p38 MAP kinase signaling pathways (2-4). Various forms of cellular stress, including genotoxic agents, inflammatory cytokines and UV irradiation, stimulate the transcriptional activity of ATF-2. Cellular stress activates ATF-2 by phosphorylation of Thr69 and Thr71 (2-4). Both SAPK and p38 MAPK have been shown to phosphorylate ATF-2 at these sites in vitro and in cells transfected with ATF-2. Mutations of these sites result in the loss of stressinduced transcription by ATF-2 (2-4). In addition, mutations at these sites reduce the ability of E1A and Rb to stimulate gene expression via ATF-2 (2).

**Description:** Activating Transcription Factor 2 (ATF-2) Fusion Protein serves as a useful substrate for SAPK/JNK and p38 MAP kinases. It is expressed as a recombinant protein fusion containing ATF-2 residues 19-96. It contains the amino-terminal activation domain of ATF-2, which is regulated by phosphorylation of Thr69 and Thr71.

**Source/Purification:** Cloned from a human cDNA library (5) and overexpressed in E. coli.

**Quality Control:** The purified protein was identified by SDS-PAGE and western blot analysis, using ATF-2 Antibody #9222, to be greater than 90% ATF-2 Fusion Protein.





Figure 2. ATF2 fusion protein was used as substrate to measure p38 kinases activity in a radiometric assay using the following reaction conditions: 25 mM Tris-HCI (pH7.5), 10 mM MgCl<sub>2</sub>, 5 mM  $\beta$ -glycerophosphate, 0.1 mM Na<sub>2</sub>VO<sub>4</sub>, 2 mM DTT, 50  $\mu$ M ATP, Substrate: ATF2 fusion protein 400 ng/ $\mu$ L, and p38 kinases: 100 ng/25  $\mu$ L.

**Directions for Use:** ATF-2 Fusion Protein, at a concentration of 2  $\mu$ g/20  $\mu$ l reaction, can be phosphorylated by an upstream kinase in an *in vitro* kinase assay with 1X Kinase Buffer (#9802) and 200  $\mu$ M ATP (#9804). After a 30-minute assay at 30°C, phosphorylation can be detected by Western blot with Phospho-ATF-2 (Thr71) Antibody (#9221).

Molecular Formula: Molecular Weight: 34 kDa

Storage: Supplied in 20 mM Tris-HCI (pH 7.5 at 25°C), 50 mM NaCI, 2 mM Na2EDTA, 1 mM dithiothreitol (DTT) and 50% glycerol. Store at  $-20^{\circ}$ C.

#### **Background References:**

- (1) Abdel-Hafiz, H.A. et al. (1992) *Mol. Endocrinol.* 6, 2079–2089.
- (2) Gupta, S. et al. (1995) Science 267, 389-393.
- (3) van Dam, H. et al. (1995) EMBO J. 14, 1798-1811.
- (4) Livingstone, C. et al. (1995) EMBO J. 14, 1785-1797.



Figure 1. Western blot analysis of ATF2 fusion protein phosphorylated by different isoforms of p38 kinase, using ATF-2 (20F1) Rabbit mAb #9226 (upper panel) and Phospho-ATF-2 (Thr71) (11G2) Rabbit mAb #5112 (lower panel). MSDS

# Material Safety Data Sheet (MSDS) for Fusion Proteins



rev. 05/16/08

# I. Identification:

Product name: Fusion Proteins Product Catalog Number: 6000, 7000, and 9000 series CAS number: None

Manufacturer Supplier: Cell Signaling Technology

3 Trask Lane Danvers, MA 01923 USA 1-978-867-2300 TEL 1-978-867-2400 FAX 1-978-578-6737 Emergency Phone

## II. Composition/Information on Ingredients:

This product is composed of proteins in aqueous buffer solution. According to 29 CFR 1910.1200(d), hazardous ingredients at less than <1% and carcinogens at less than < 0.1% are considered non-hazardous. This product may contain the following hazardous ingredients:

Ingredient	CAS#	Percent
Glycerol	56-81-5	50%

## **III. Hazard Identification:**

To the best of our knowledge, the chemical, physical, and toxicological properties of this solution have not been thoroughly investigated.

Emergency Overview of Hazardous ingredient: Glycerol (CAS# 56-81-5)

**Caution:** Avoid contact and inhalation. **Target organ:** Kidneys.

## **IV. First Aid Measures:**

Inhalation: If inhaled, remove to fresh air. If breathing is difficult, get medical attention. Ingestion: If swallowed, rinse mouth with water provided person is conscious. Get medical attention.

Skin Exposure: In case of contact, wash skin with soap and water.

**Eye Exposure:** In case of contact with eyes, immediately flush eyes with water for at least 15 minutes. Get medical attention.

## V. Fire Fighting Measures:

Flash Point: Data not available.

Autoignition Temperature: Data not available.

**Fire Extinguishing Media:** Water spray, dry chemical, foam, or carbon dioxide. **Firefighting:** Wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes.

## VI. Accidental Release Measures:

Absorb liquid with an absorbent material. Transfer contaminated absorbent to a chemical waste container for disposal.

## VII. Handling And Storage:

Avoid inhalation and contact with eyes and skin. Avoid prolonged or repeated exposure. Store at–20°C in tightly closed container.

## VIII. Exposure Controls/Personal

Engineering Controls: Maintain adequate ventilation, eye wash and quick-drench facilities in work area.

Personal Protective Equipment: Lab coat, chemical resistant gloves and chemical safety glasses.

Occupational Exposure Limits: Data not available.

## IX. Exposure Controls/Personal Protection:

Physical State: Appearance: Odor: Boiling Point: Melting Point: Volatile Organic Compound: Solubility in water: liquid colorless odorless data not available data not available data not available readily miscible in water

## X. Stability and Reactivity:

Stability: Stable under recommended conditions. Hazardous Decomposition: May form carbon dioxide and carbon monoxide. Conditions to avoid: Strong oxidizing agents.

#### XI. Toxicological Information:

May cause skin irritation. May be toxic if absorbed through skin or ingested. May cause eye irritation.

#### Target Organs: Kidneys

Prolonged exposure may cause nausea, headache, and vomiting.

## XII. Ecological Information:

Data not available.

#### XIII. Disposal Considerations:

Dispose of in accordance with federal, state and local environmental regulations.

## **XIV. Transport Information:**

**D.O.T.:** This substance is considered non-hazardous for transport. **IATA:** This substance is considered non-hazardous for air transport.

## XV. Regulatory Information:

US Classification and Label information:

**Caution:** Avoid contact and inhalation. **Target organ(s):** Kidneys.

Chemical inventory status : Not classified/controlled according to EU, USA, WHMIS.

## XVI. Other Information:

This product is not intended for use in humans. It is sold only for research use only. No other use is intended, and any other use may involve substantive hazards.

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide for experienced personnel. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product. The burden of safe use of this material rests entirely with the user.