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Product Information

ATF-2 (Activating transcription factor 2), human recombinant, expressed in *E. coli*

Catalog Number **A2353**
Storage Temperature –20 °C

Synonyms: ATF-2 (AA 20-109), Maltose binding fusion protein (MBP); CRE-BP-1 (cAMP-responsive element binding protein-1)

Product Description
ATF-2 is a member of the leucine zipper protein family of transcription factors which regulates gene transcription by interacting with ATF/cAMP-response elements of genes.[1] The transcriptional activity of ATF-2 is stimulated by phosphorylation of its amino acid residues Thr^{69} and Thr^{71}, carried out by stress activated protein kinases (SAPKs) including JNK and p38 MAP kinase.[2]

This human, recombinant ATF-2 product (amino acids 20–109) is expressed in *E. coli* as a N-terminal maltose binding fusion protein (MBP). It is provided as a lyophilized powder containing Hepes buffered salts, NaCl, DTT, EGTA, Brij® 35, trehalose, and protease inhibitors.

Apparent molecular mass: 53 kDa

Purity: ≥90% (SDS-PAGE)

This product is tested as a substrate for p38 MAP kinase (p38 MAPK).

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 the product at –20 °C. Avoid repeated freezing and thawing to prevent denaturing the protein. Do not store in frost-free freezers.

Procedure
Sample preparation
ATF2 solution – Reconstitute the ATF2 with ultrapure water to a concentration of 1 mg/mL. Refer to the Certificate of Analysis for the protein concentration and reconstitution instructions. To obtain CofA go to www sigma-aldrich.com

Reagent preparation
Reaction Buffer (RB) – 75 mM β-glycerophosphate, pH 7.3, 30 mM $MgCl_2$, 4.5 mM DTT, 0.15 mM Na-vanadate, and 3.75 mM EGTA. Keep the solution at –20 °C.

Enzyme Dilution Buffer (EDB) – 50 mM β-glycero-phosphate, pH 7.3, 1 mM DTT, 0.1 mM Na-vanadate, 1.5 mM EGTA, and 1 mM EDTA. Keep the solution at –20 °C.

0.5% Phosphoric acid in ultrapure water.

Phosphocellulose paper – cut into 1.5 × 6 cm pieces. Mark the tube number on the paper pieces with a pencil.

γ-^{32}P-ATP-Reaction Buffer – Add 1 µL of γ-^{32}P-ATP (10 mCi/mL) per 100 µL of Reaction Buffer (RB). For each assay prepare 12 µL of the γ-^{32}P-ATP–RB solution. Prepare enough solution for the planned number of assays, plus 3 additional assays.

MKK6 solution – Dilute MKK6 enzyme (Catalog Number M5814) with EDB to 12.5 units/mL.

p38 MAPK solution – Dilute p38 MAPK to a concentration of 50 µg/mL with EDB.

Kinase Assay

The 2-step assay measures ATF2 phosphorylation by p38 MAPK. In the first step the p38 MAPK is phosphorylated by MKK6 to yield active p38 MAPK. The second step measures p38 MAPK activity (phosphorylation) on the ATF2 substrate.

This assay involves the use of the ^{32}P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Pipette 4 µL of p38 MAPK solution (50 µg/mL) and 10 µL of the MKK6 solution (125 millunits total) into a microcentrifuge tube.
2. Pipette 4 µL of p38 MAPK solution and 10 µL of EDB into a second microcentrifuge tube (Blank 1 – no MKK-6).
3. Pipette 10 µL of the MKK6 and 4 µL of EDB into a third tube (Blank 2 – no p38 MAPK).
4. Add 12 µL of the γ-^{32}P-ATP–RB solution to all the tubes. Vortex gently, for a few seconds, and incubate for 30 minutes at 30 °C.
5. Add 10 µL of ATF2 solution (1 mg/mL) to the tubes.
6. Vortex gently, for a few seconds, and incubate for another 5 minutes at 30 °C.
7. Apply 25 µL from each reaction mixture to a piece of phosphocellulose P-81 paper (1.5 × 6 cm) and soak the pieces in 0.5% Phosphoric acid.
8. Wash the pieces of the phosphocellulose paper 4 times with 0.5% Phosphoric acid. For each wash agitate gently for 5–6 minutes.
9. Wash once with ethanol for 1 minute.
10. Wash once with acetone for 1 minute.
11. Dry the pieces of paper at room temperature or under a heat lamp, and count the radioactivity incorporated using Cerenkov mode (i.e., count the β-emission without scintillation liquid, using the tritium channel).

Assay scheme

Step I	**Blank 1**	**Blank 2**	**Sample**
p38 MAPK	4 µL	–	4 µL
EDB	10 µL	4 µL	–
MKK6 solution	–	10 µL	10 µL
RB	12 µL	12 µL	12 µL
Incubate 30 minutes at 30 °C			
Step II			
ATF2 solution	10 µL	10 µL	10 µL
Incubate 5 minutes at 30 °C			

12. Set up a radioactive control to measure the total γ-^{32}P-ATP counts introduced into the reaction. Spot 12 µl of the γ-^{32}P-ATP–RB on a precut phosphocellulose strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample. Perform in duplicates.

Results

Calculation of the p38 MAPK specific Activity
(nmole/min/mg = units/mg)

$$\frac{C}{SR \times \text{time} \times \text{mg protein}} = \text{nmole/min/mg} = \text{units/mg}$$

$$\frac{C}{SR \times 5 \times 0.0002} = \text{nmole/min/mg} = \text{units/mg}$$

Total counts (**R**, radioactivity introduced into each reaction tube) – cpm value from radioactive control (step 12).

Specific radioactivity (**SR**) – divide the total counts (**R**) by the amount of ATP present in the assay tube (3.6 nmole), **SR** = R/3.60 (cpm/nmole).

Total counts of each sample (**C**) – adjust counts (cpm) of 25 µL spotted on paper to 36 µL of total sample, **C** = 36/25 (1.44).

Time – 5 minutes

mg of protein – 0.0002 mg (0.2 µg) p38 MAPK protein in the assay

References

1. Herdegen, T., and Leah, J.D., Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Res. Rev., **28**, 370-490 (1998).
2. Livingstone, C. et al., ATF-2 contains a phosphorylation-dependent transcriptional activation domain. EMBO J., **14**, 1785-1797 (1995).

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