BioVision
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p38β, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # 7763-5, -100

Purity:	>90%
Storage:	-80°C
Shipping:	in Dry ice
Shelf Life:	6-12 months from shipping date
Aliquot Size:	5 μg and 100 μg
Concentration:	0.1 μg/μl
Specific Activity:	**123 nmol/min/mg**

Product Description

Recombinant full-length human p38beta was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_002751.

Gene Aliases

MAPK11; SAPK2; p38-2; PRKM11; SAPK2B; p38b; P38b2

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, and 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

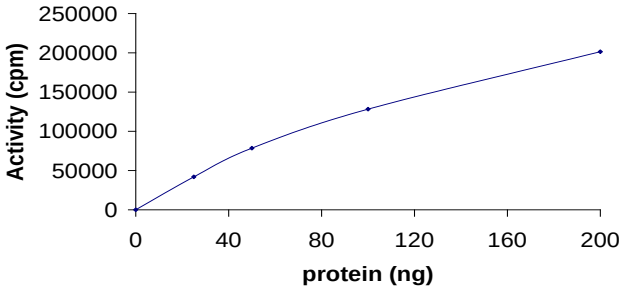
Scientific Background

p38-beta is a member of the p38 MAP kinase family and is activated by both proinflammatory cytokines and environmental stress (1). The p38-beta is activated through its phosphorylation by MAP kinase kinases (MKKs), preferably by MKK6. Transcription factor ATF2/CREB2 has been shown to be a substrate of this kinase (2). Alternatively spliced transcript variants encoding the same protein have been observed.

References

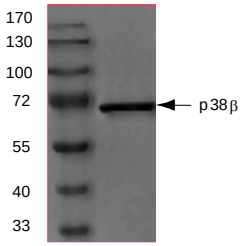
1. Jiang, Y. et al: Characterization of the structure and function of a new mitogen-activated protein kinase (p38-beta). *J. Biol. Chem.* 271: 17920-17926, 1996.
2. Stein, B. et al: p38-2, a novel mitogen-activated protein kinase with distinct properties. *J. Biol. Chem.* 272: 19509-19517, 1997.

Specific Activity



The specific activity of P38BETA was determined to be **123 nmol/min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry, Approx. MW **71kDa**.

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Activity Assay Protocol

Reaction Components

Active Kinase

Active p38beta (0.1μg/μl) diluted with Kinase Dilution Buffer III and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active p38beta for optimal results).

[^{32}P]-ATP Assay Cocktail

Prepare 250μM [^{32}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution, 100μl [^{32P}]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer. Store 1ml aliquots at –20°C.

Kinase Dilution Buffer, pH 7.2

Kinase Assay Buffer I diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

10mM ATP Stock Solution

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer. Store 200μl aliquots at –20°C.

Kinase Assay Buffer I, pH 7.2

Buffer components: 25mM MOPS, 12.5mM β-glycerol-phosphate, 25mM $MgCl_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

Substrate

ATF2 substrate prepared in buffer (50mM Tris-HCl, pH 7. 2, 50mM $NaCl_2$, 5mM EDTA and 0.25mM DTT) to a final concentration of 0.5mg/ml.

Assay Protocol

Step 1. Thaw [^{32}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active p38beta, Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

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Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5μl [^{32}P]-ATP Assay Cocktail bringing the final

Step 6. volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 7. After the 15 minute incubation period, terminate the reaction by spotting 20μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 8. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 9. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 10. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P^{32}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5μl [^{32}P]-ATP / pmoles of ATP (in 5μl of a 250μM ATP stock solution, i.e., 1250pmoles)

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{32}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]