

MYO3 β , Active

Recombinant protein expressed in Sf9 cells

Catalog # **M66-11G**

Lot # C055-2

Product Description

Recombinant human MYO3 β (1-326) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_138995](#).

Gene Aliases

myosin IIIB

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 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.

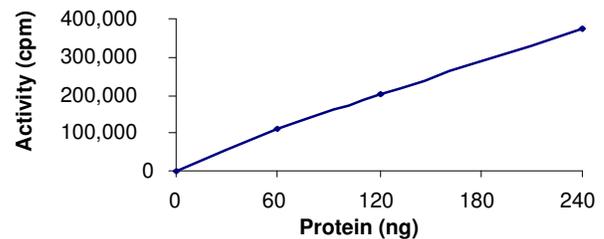
Scientific Background

MYO3 β is a member of the Class III myosins that are actin-dependent motor proteins containing an amino-terminal kinase domain. MYO3 β contains an N-terminal kinase domain, followed by motor, neck, and tail domains. The MYO3 β gene generates a variety of splice variants that contain 1 or 2 calmodulin-binding (IQ) motifs in the neck domain and 1 of 3 domains in the tail domain. Northern blot analysis shows expression of a 7-kb MYO3 β transcript in the human retina but not in a RPE cell line (1). The MYO3 β gene transcript is also detected in the kidney, intestine and testis. The MYO3 β gene maps to chromosome 2q31.1-q31.2 by genomic sequence alignment.

References

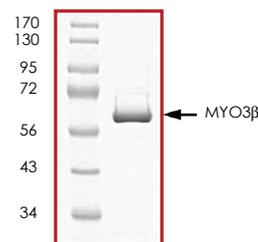
1. Dose, A C. et al: A class III myosin expressed in the retina is a potential candidate for Bardet-Biedl syndrome. *Genomics* 79: 621-624, 2002.

Specific Activity



The specific activity of MYO3 β was determined to be **85 nmol /min/mg** as per activity assay protocol.

Purity



The purity of MYO3 β was determined to be **>90%** by densitometry, approx. MW **63kDa**.

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Specific Activity

85 nmol/min/mg

Specific Lot Number

C055-2

Purity

>90%

Concentration

0.1 $\mu\text{g}/\mu\text{l}$

Stability

1yr at -70°C from date of shipment

Storage & Shipping

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. Product shipped on dry ice.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: M66-11G)

Active MYO3 β (0.1 μ g/ μ l) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) 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 MYO3 β for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled H₂O.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β -glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³²P]-ATP Assay Cocktail

Prepare 250 μ M [³²P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 μ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 μ l [³²P]-ATP (1mCi/100 μ l), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200 μ l aliquots at -20°C.

Substrate (Catalog #: M42-54G)

Myelin Basic Protein (MBP) diluted in distilled H₂O to a final concentration of 0.2mg/ml.

Assay Protocol

- Step 1.** Thaw [³²P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active MYO3 β , Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 μ l:
 - Component 1.** 10 μ l of diluted Active MYO3 β (Catalog #M66-11G)
 - Component 2.** 5 μ l of 0.2mg/ml stock solution of substrate (Catalog #M42-54G)
 - Component 3.** 5 μ l distilled H₂O (4°C)
- 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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5 μ l [³²P]-ATP Assay Cocktail bringing the final volume up to 25 μ l and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 μ l of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** 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 H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** 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 [³²P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μ l [³²P]-ATP / pmoles of ATP (in 5 μ l of a 250 μ M ATP stock solution, i.e., 1250 pmoles)

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

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

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