

# MYO3B Kinase

☑ 5 µg



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This product is for *in vitro* research use only and is not intended for use in humans or animals.

**Description:** Purified recombinant human MYO3B (Met1-Glu326) kinase, supplied as a GST fusion protein.

**Background:** Class III myosins comprise one group of at least 18 classes of the myosin superfamily. All myosin motor proteins share a relatively conserved N-terminal motor domain that contains nucleotide and actin binding sites, a calmodulin-binding consensus (IQ) motif found within the neck domain and more variable and class-specific tail domains for cargo binding and trafficking. Class III myosins are the only actin-based motor proteins that have intrinsic protein kinase activity (3) as they have an N-terminal kinase domain attached to the myosin head (1,2). A pair of myosin III proteins has been identified to date. MYO3A is expressed predominantly in the retina and retinal pigmented epithelium, while MYO3B is found in the retina, testis and kidney. MYO3A is implicated in photoreceptor function while the gene corresponding to MYO3B lies in a region associated with Bardet-Biedl syndrome, a disorder characterized in part with retinal dystrophy (1,2).

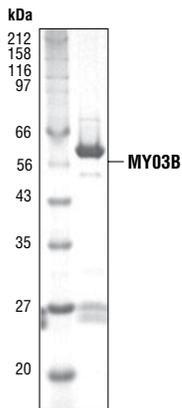


Figure 1. The purity of the GST-MYO3B fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human MYO3B (Met1-Glu326) (GenBank Accession No. NM\_138995) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

**Quality Control:** The theoretical molecular weight of the GST-MYO3B fusion protein is 63 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. MYO3B kinase activity was determined using a radiometric assay [Fig.2].

**Background References:**

- (1) Dosé, A.C. and Burnside, B. (2002) *Genomics* 79, 621–4.
- (2) Komaba, S. et al. (2003) *J Biol Chem* 278, 21352–60.
- (3) Dosé, A.C. and Burnside, B. (2000) *Genomics* 67, 333–42.

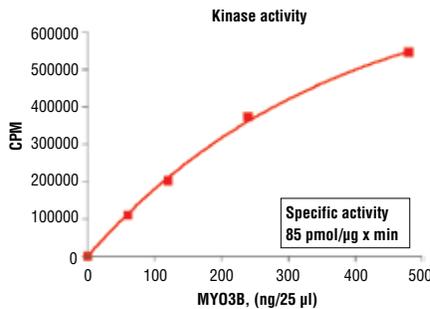


Figure 2. MYO3B kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM MgCl<sub>2</sub>, 0.05 mM DTT, 50 µM ATP, Substrate: MBP 200 ng/µL, and variable amounts of MYO3B kinase.

**Storage:** Enzyme is supplied in 50 mM Tris-HCl, pH7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione.

Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

**Companion Products:**

Serine/Threonine Kinase Substrate Screening Kit #7400

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

# Protocol for MYO3B Kinase Assay

**Note:** Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

## A Additional Solutions and Reagents (Not included)

### 1. Kinase Buffer (10X)

50 mM MOPS, pH 7.2  
25 mM  $\beta$ -glycerophosphate  
10 mM EGTA  
4 mM EDTA  
50 mM  $MgCl_2$   
0.5 mM DTT

2. ATP (10 mM) #9804

3.  $^{32}P$ -ATP

4. Myelin Basic Protein (MBP) (0.5  $\mu$ g/ $\mu$ l)

## B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250  $\mu$ M ATP.
2. Dilute [ $^{32}P$ ] ATP to 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP with 250  $\mu$ M ATP solution.
3. Transfer enzyme from  $-80^\circ C$  to ice. Allow enzyme to thaw on ice.
4. Dilute MYO3B kinase protein (100 ng/ $\mu$ l concentration) to 20 ng/ $\mu$ l with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10  $\mu$ l diluted MYO3B kinase solution, 10  $\mu$ l MBP (0.5  $\mu$ g/ $\mu$ l), and 5  $\mu$ l 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP solution.

## Final Assay Conditions

5 mM MOPS, pH 7.2  
2.5 mM  $\beta$ -glycerophosphate  
1 mM EGTA  
0.4 mM EDTA  
4 mM  $MgCl_2$   
0.05 mM DTT  
200 ng/ $\mu$ l MBP

6. After 15 minutes terminate reaction by spotting 20  $\mu$ l of the reaction mixture onto phosphocellulose P81 paper.
7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
9. Count samples in a scintillation counter.

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