V. 1.1

Heavy Meromyosin Protein
(Rabbit skeletal muscle)
Cat. # MH01

Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Material

Heavy meromyosin has been produced by α-chymotrypsin proteolytic cleavage of full-length myosin II protein isolated from rabbit skeletal muscle. Heavy meromyosin consists of an active motor fragment consisting of two head domains connected by their subfragment-2 (S2) regions and two pairs of light chains, essential light chain (ELC) and regulatory light chain (RLC), see Figure 2. Heavy meromyosin has been determined to be biologically active in an F-actin activated ATPase assay (see below). Heavy meromyosin is supplied as a white lyophilized powder.

Storage and Reconstitution

Briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 5 mg/ml by the addition of 10 µl of Milli-Q water. The protein will be in the following buffer; 10 mM imidizole pH 7.0, 50 mM KCl, 3 mM $MgCl_2$, 1 mM DTT, 5% (w/v) sucrose and 1% (w/v) dextran. In order to maintain high biological activity of the protein, it is recommended that the protein solution be aliquoted into "experiment sized" amounts, snap frozen in liquid nitrogen and stored at -70°C. The protein is stable for 6 months if stored at -70°C. The protein should not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable at 4°C desiccated (<10% humidity) for 1 year.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% gradient polyacrylamide gel. Heavy meromyosin protein was determined to be 70% pure (see Figure 1).

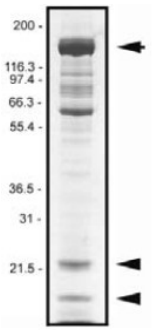

Figure 1. Heavy Meromyosin Protein Purity Determination. A 10 µg sample of rabbit skeletal heavy meromyosin protein was separated by electrophoresis in a 4-20% SDS-PAGE system, and stained with Coomassie Blue. Arrow indicates the truncated myosin head domain (approx. 150 kDa), arrowheads indicate the RLC (approx. 20 kDa) and ELC (approx. 17 kDa). Protein quantitation was performed using the Precision Red™ Protein Assay Reagent (Cat.# ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay

The biological activity of rabbit heavy meromyosin can be determined from its rate of F-actin activated ATP hydrolysis. A standard biological assay for monitoring ATP hydrolysis by heavy meromyosin consists of an in vitro F-actin ATPase assay (1). Stringent quality control ensures that in the presence of F-actin, rabbit heavy meromyosin will have a minimum hydrolysis rate 500 fold greater than in the absence of F-actin. In the assay described below the ATP hydrolysis rate for MH01 is in the range of 500—600 nmol ATP hydrolysed per minute per mg of heavy meromyosin protein.

Reagents

1. Rabbit skeletal heavy meromyosin (50 µg, Cat. # MH01)
2. Pre-formed F-actin Filaments (rabbit skeletal muscle, 1 mg, Cat. # AKF99)
3. CytoPhos™ Phosphate Assay Biochem Kit™ (Cat. # BK054)
4. 100 mM ATP in 50 mM Tris-HCl pH 7.5 (Made fresh just prior to use)
5. 15 mM Tris-HCl pH 7.5
6. Reaction buffer (15 mM Tris HCl, pH 7.5, 2.5 mM $MgCl_2$, 0.1 mM EGTA)

Equipment

1. Spectrophotometer capable of measuring absorbance at 650 nm. We recommend a SpectraMAX250 (Molecular Devices)
2. Half area 96 well microtiter plate
3. Multi-channel pipettor

Method

1. Resuspend the pre-formed F-actin filaments to 0.4 mg/ml with 2.4 ml of room temperature Milli-Q water.
2. Dilute heavy meromyosin (Cat. # MH01) to 0.05 mg/ml with reaction buffer. Keep on ice.
3. Just before use, dilute the 100 mM ATP stock to 4 mM in 15 mM Tris-HCl pH 7.5. Keep on ice.
4. Add the following components to duplicate control wells: 7 µl reaction buffer and 15 µl 0.4 mg/ml F-actin (final concentration will be 0.2 mg/ml in a 30 µl reaction volume).
5. Add the following components to duplicate experiment wells: 1 µl reaction buffer, 15 µl 0.5 mg/ml F-actin and 6 µl 0.05 mg/ml heavy meromyosin (final concentration will be 0.01 mg/ml in a 30 µl reaction volume).
6. Using a multichannel pipettor immediately add 8 µl of 4 mM ATP to each well simultaneously to start the ATPase reaction (final concentration will be 1 mM in a 30 µl reaction volume).
7. Briefly mix the components and incubate the plate at 37°C

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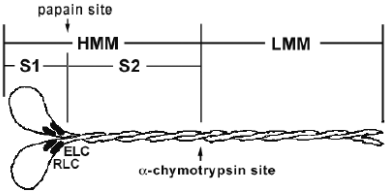
for exactly 5 min.

8. Terminate the reaction by adding 70 µl of CytoPhos™ reagent to each well. Incubate at room temperature for 10 min. Refer to the BK054 instruction manual for additional information.
9. Read the absorbance at 650 nm in the spectrophotometer.
10. Vmax measurements are calculated with use of a standard phosphate curve as described in the PhosFree phosphatase assay (Cat. # BK054).

Figure 2. Diagrammatic representation of the myosin II protein and its subfragments. Myosin II or conventional myosin is a hexameric protein consisting of two heavy chains and two light chains. Myosin II can be proteolytically cleaved into heavy meromyosin (HMM) and light meromyosin(LMM) by α-chymotrypsin. Heavy meromyosin consists of the myosin head subfragment-1 domain (S1), its associated light chains (essential light chains and regulatory light chains), and the coiled-coil subfragment -2 domain. Light meromyosin consists of coiled-coil protein structure. The myosin S1-subfragment is produced by papain digestion of HMM.



Product Uses

- Measurement of F-actin activated ATPase activity
- Identification/characterization of proteins or small molecules that affect heavy meromyosin ATPase activity
- Identification/characterization of proteins or small molecules that affect heavy meromyosin F-actin interaction

References

1. Kodama, T., et al. 1986. J. Biochem. 99, 1465-1472.

Product Citations/Related Products

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