V. 3.0

Myosin Motor Protein (full length)
(Bovine Cardiac Muscle)
Cat. # MY03

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

Material

Myosin protein has been purified from bovine cardiac muscle (1, 2). The full length myosin protein has been purified with its essential light chains (ELC) and regulatory light chains (RLC), see Figure 1 and 2. Myosin has been determined to be biologically active in an F-actin activated ATPase assay (see biological activity assay). Bovine cardiac myosin protein is supplied as a white lyophilized powder.

Storage and Reconstitution

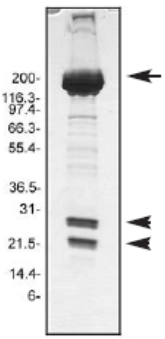
Briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 10 mg/ml by the addition of 100 µl of Milli-Q water. The protein will be in the following buffer: 25 mM PIPES pH 7.0, 1.25 M KCl, 2.5% (w/v) sucrose and 0.5% (w/v) dextran. In order to maintain 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. Myosin protein was determined to be 90% pure (see Figure 1).

Figure 1. Myosin Protein Purity Determination.

A 10 µg sample of bovine cardiac myosin protein was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Arrow indicates the myosin heavy chain (approx. 200 kDa), arrowheads indicate the RLC (approx. 20 kDa) and two ELC isoforms (approx. 25 and 21 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 bovine cardiac myosin can be determined from its rate of F-actin activated ATP hydrolysis. A standard biological assay for monitoring ATP hydrolysis by myosin consists of an in vitro F-actin stimulated myosin ATPase assay (1). Stringent quality control ensures that in the presence of F-actin, bovine cardiac myosin will have a minimum ATP hydrolysis rate 10 fold greater than in the absence of F-actin and a minimum actin activated ATPase activity >25 nmol/min/mg of myosin under the experimental conditions given below.

Reagents

1. Bovine cardiac myosin (1 mg, Cat. # MY03)
2. Preformed F-actin filaments (Cat. # AKF99)
3. CytoPhos™ Phosphate Assay Biochem Kit (Cat. # BK054)
4. 100 mM ATP in 50 mM Tris-HCl pH 7.5
5. 15 mM Tris-HCl pH 7.5
6. Reaction buffer (15 mM Tris HCl, pH 7.5, 10 mM KCl, 2 mM $MgCl_2$, 0.1 mM EGTA)
7. Myosin Resuspension buffer (0.2M KCl, 1 mM $MgCl_2$, 15 mM Tris HCl, pH 7.5)

Equipment

1. Spectrophotometer capable of measuring absorbance at 650 nm. We recommend a SpectraMAX250 (Molecular Devices)
2. Half area 96 well microtiter plate (Corning Cat.# 3696 or 3697)
3. Multi-channel pipette

Method

1. Resuspend preformed F-actin filaments (Cat. # AKF99) to 1 mg/ml with reaction buffer.
2. Resuspended the bovine cardiac myosin (Cat. # MY03) to 0.1 mg/ml with 10 ml of Myosin Resuspension buffer. Incubate the protein on ice for 5 min for complete resuspension.
3. Add the following components to duplicate **actin control** wells: 13 µl reaction buffer, 12 µl F-actin.
4. Add the following components to duplicate **myosin control** wells: 23 µl reaction buffer, 2 µl of myosin working stock .
5. Add the following components to duplicate **experiment** wells in the order shown: 11 µl reaction buffer, 12 µl of F-actin and 2 µl of myosin.
6. Just before use, dilute the 100 mM ATP stock to 3 mM in 15 mM Tris-HCl pH 7.5. Keep on ice.
7. Using a multichannel pipette, add 5 µl of ATP to each well simultaneously to start the ATPase reaction.
8. Briefly mix the components and incubate the plate at 37°C for exactly 60 min.

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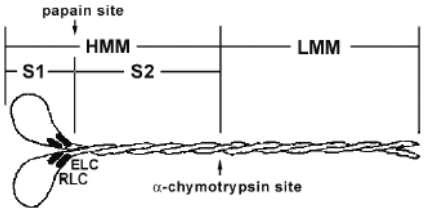
9. Terminate the reaction by adding 70 µl of CytoPhos™ reagent to each well.
10. Incubate at room temperature for 10 min.
11. Read the absorbance at 650 nm in the spectrophotometer.
12. The nmoles of free phosphate (Pi) generated from ATP hydrolysis in each reaction can be determined by comparing Pi values generated with a standard phosphate curve (described in Cat.# BK054).
13. The F-actin activated myosin ATPase activity should be >25 nmoles of Pi generated per minute per mg of myosin.

Figure 2. Diagrammatic representation of the myosin protein and its subfragments



Myosin is a hexameric protein consisting of two heavy chains and two light chains. Myosin can be proteolytically cleaved into heavy meromyosin and light meromyosin 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 myosin ATPase activity
- Identification/characterization of proteins or small molecules that affect myosin ATPase activity
- Identification/characterization of proteins or small molecules that affect myosin / F- actin interaction

References

1. Pollard, T.D., . 1982. Methods in Cell Biol. 24:333
2. Margossian, S.S., and Lowey, S. 1982. Methods in Enzymology. 85:55-71.

Product Citations/Related Products

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