Myosin Motor Protein (full length)
(Rabbit skeletal muscle)
Cat. # MY02
5 x 1 mg

V. 4.0

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

Material

Myosin II protein has been purified from rabbit skeletal muscle (1, 2). The full length myosin II protein has been purified with its essential light chains (ELC) and regulatory light chains (RLC), see Figure 1 and 2. Myosin II has been determined to be biologically active in an F-actin activated ATPase assay (see biological activity assay). Rabbit skeletal muscle myosin II is not recommended for use in motility assays. Rabbit myosin II protein is supplied as a white lyophilized powder.

Storage and Reconstitution

The protein should be reconstituted to 10 mg/ml by the addition of 100 µl of Milli-Q water containing 1 mM DTT. Once 1 mM DTT has been added the myosin should be left at room temperature for 1-2h with occasional gentle tapping to disperse the myosin powder. After 1-2h the myosin can be pipetted gently up and down 3-4 times with a pipette set at 100 µl. Avoid excessive pipetting and the introduction of air bubbles as myosin is extremely sensitive to oxidation which will inactivate the protein. The protein will be in the following buffer: 25 mM PIPES pH 7.0, 1.25 M KCl, 1 mM DTT, 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 used immediately. For longer term storage the protein can 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).

Biological Activity Assay

The biological activity of rabbit myosin II 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 ATPase assay (1). Stringent quality control ensures that in the presence of F-actin, rabbit myosin will have a minimum hydrolysis rate 10 fold greater than in the absence of F-actin and a minimum actin activated ATPase activity >300 nmol/min/mg of myosin under the experimental conditions given below.

Reagents

1. Rabbit skeletal myosin (1 mg, Cat. # MY02)
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 (25 mM Tris HCl, pH 7.5, 35 mM KCl, 0.1 mM EGTA, 1 mM $MgCl_2$)
7. Myosin Resuspension buffer (15 mM Tris HCl, pH 7.5, 0.2 M KCl, 1 mM $MgCl_2$)

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. Resuspend rabbit myosin II (Cat.# MY02) to 0.1 mg/ml with Myosin resuspension buffer.
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 myosin protein.
5. Add the following components to duplicate experiment wells: 11 µl reaction buffer, 12 µl F-actin, and 2 µl myosin.
6. Just prior to use, dilute the 100 mM ATP stock to 3 mM in 15 mM Tris-HCl pH 7.5.
7. Use a multichannel pipette to add 5 µl of ATP to each well simultaneously to start the reaction.
8. Briefly mix the components and incubate the plate at 37°C for exactly 10 minutes.
9. Terminate the reaction by adding 70 µl of CytoPhos™ reagent to each well.
10. Incubate at room temperature for 10 minutes.
11. Read the absorbance at 650 nm in the spectrophotometer.
12. The nmoles of Pi generated from each reaction can be determined by comparing Pi values generated with a standard phosphate curve (described in Cat.# BK054).
13. The F-actin stimulated myosin ATPase activity should be >300 nmoles of Pi generated per minute per mg of myosin.

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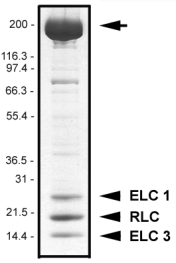
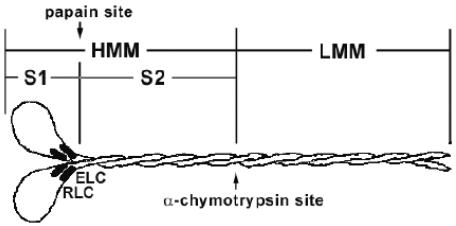
Figure 1. Myosin Protein Purity Determination



A 20 ug sample of rabbit skeletal myosin II 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 17 kDa). Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat.# ADV02). Mark12 molecular weight markers are from Invitrogen.

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, two essential light chains (ELCs), and two regulatory light chains (RLCs). Myosin II can be proteolytically cleaved into heavy meromyosin (HMM, Cat.# MH01) 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 myosin ATPase activity
- Identification/characterization of proteins or small molecules that affect myosin II ATPase activity
- Identification/characterization of proteins or small molecules that affect myosin II 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.

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