

**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 below). Bovine cardiac myosin protein is supplied as a white lyophilized powder.

**Table 1. Actin Activated Myosin ATPase Activity**

MY03	ATPase activity minus F-actin (nmol/min/mg)	ATPase activity plus F-actin (nmol/min/mg)
Lot 5	0 +/- 0.04	25

**Storage and Reconstitution**

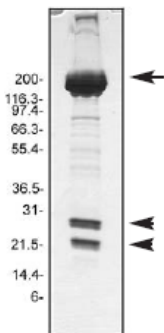
Briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 20 mg/ml by the addition of 50 µl of Milli-Q water. The protein will be in the following buffer: 3.0 M KCl, 5% (w/v) sucrose and 1% (w/v) dextran. In order to maintain high biological activity 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. 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 ATPase assay (1). Stringent quality control ensures that in the presence of F-actin, bovine cardiac myosin will have a minimum hydrolysis rate 10 fold greater than in the absence of F-actin (see Table 1).

**Reagents**

1. Bovine cardiac myosin (1 mg, Cat. # MY03)
2. Performed F-actin filaments (bovine cardiac muscle, 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 (20 mM Tris HCl, pH 7.5, 15 mM KCl, 6 mM MgCl<sub>2</sub>, 2 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 (Corning Cat.# 3696 or 3697)
3. Multi-channel pipettor

**Method**

1. Resuspend preformed F-actin filaments (Cat. # AKF99) to 0.4 mg/ml with 2.4ml Milli-Q water.
2. Resuspended the bovine cardiac myosin (Cat. # MY03) to 2 mg/ml with 500 µl of cold Milli-Q water. Incubate the protein on ice for 5 min for complete resuspension. Centrifuge for 5 min at 12k rpm. Gently remove the supernatant to a clean tube and keep on ice.
3. Add the following components to duplicate **actin control** wells: 10 µl reaction buffer, 12 µl 0.4 mg/ml F- actin (final concentration will be 0.2 mg/ml in a 30 µl reaction volume).
4. Add the following components to duplicate **myosin control** wells: 20 µl reaction buffer, 2 µl of 2 mg/ml myosin working stock (final concentration will be 0.14 mg/ml in a 30 µl reaction volume).
5. Add the following components to duplicate **experiment** wells: 8 µl reaction buffer, 12 µl of 0.5 mg/ml F-actin and 2 µl of 2 mg/ml myosin working stock.
6. Just before use, dilute the 100 mM ATP stock to 4 mM in 15 mM Tris-HCl pH 7.5. Keep on ice.
7. Using a multichannel pipettor, add 8 µl of 4 mM ATP to each well simultaneously to start the ATPase reaction (final

8. concentration will be 1 mM in a 30  $\mu$ l reaction volume).
9. Briefly mix the components and incubate the plate at 37°C for exactly 10 min.
10. Terminate the reaction by adding 150  $\mu$ l of CytoPhos™ reagent to each well. Incubate at room temperature for 10 min.
11. Read the absorbance at 650 nm in the spectrophotometer.
12. Vmax values are calculated with use of a standard phosphate curve as described in the Cytophos Phosphate Assay kit (Cat. # BK054) using 30  $\mu$ l each of 10, 30, 60 and 100  $\mu$ M Potassium phosphate dibasic ( $K_2HPO_4$ ).
13. Assay results for this Lot of myosin is shown in Table 1.

#### Product Uses

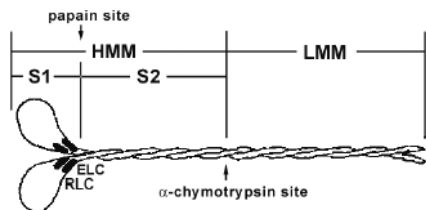
- Measurement of F-actin activated myosin ATPase activity
- Identification/characterization of proteins or small molecules that affect myosin ATPase activity
- Identification/characterization of proteins or small molecules that affect myosin / F- actin interaction

#### 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

For the latest citations and related products please visit [www.cytoskeleton.com](http://www.cytoskeleton.com).



**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  $\alpha$ -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.