V. 1.0

Myosin Motor Protein (S1 Fragment) (Bovine Cardiac Muscle)

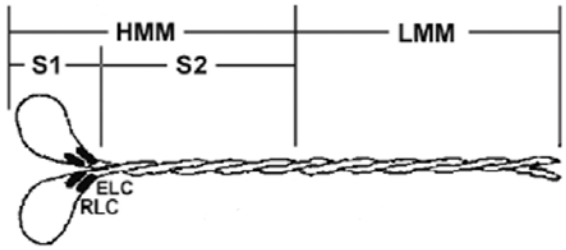
Cat. # CS-MYS03

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

Material

Cardiac myosin protein has been purified from bovine heart tissue (1, 2). The full length myosin protein was purified with its essential light chains (ELC) and regulatory light chains (RLC), see Figure 1 and 2. Myosin was then digested with α-chymotrypsin to liberate the soluble subfragment-1 (S1) domain, which was isolated by centrifugation (3). The purified myosin S1 fragment has been determined to be biologically active in an F-actin activated ATPase assay (see biological activity assay). Bovine cardiac myosin S1 fragment protein is supplied as a white lyophilized powder.

Figure 1. 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 (HMM) and light meromyosin (LMM) by α-chymotrypsin in the presence of magnesium. In the presence of EDTA, however, α-chymotrypsin produces the soluble myosin S1 fragment (3).

Storage and Reconstitution

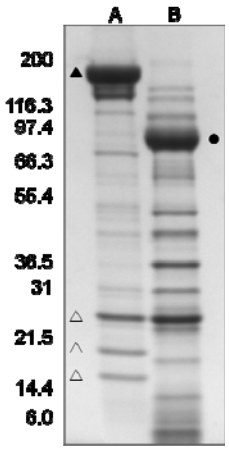
Briefly centrifuge to collect the product at the bottom of the tube. Reconstituting a 1 mg tube of MYS03 with 300 µl of Milli-Q water (3 ml for the 10 mg bottles) will generate a 3.3 mg/ml stock of cardiac S1 myosin in the following buffer: 20 mM PIPES pH 7.0, 5% (w/v) sucrose and 1% (w/v) dextran. The 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. The myosin and its light chains used to produce the myosin S1 fragment was determined to be 90% pure (see Figure 2).

Figure 2. Full length and S1 myosin.

A 20 µg sample of full length bovine cardiac myosin protein (lane A) and the corresponding S1 myosin (lane B) were separated by electrophoresis using a 4-20% SDS-PAGE gel and stained with Coomassie Blue. The 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 S1 fragment can be determined from its rate of F-actin activated ATP hydrolysis. The assay is constructed by first polymerizing actin to form F-actin, then the cardiac Tropomyosin/Troponin complex (TT complex) is mixed with the actin filaments in a stoichiometric amount. This creates coated filaments which are analogous to the thin filaments of muscle fibers. Myosin is added in substoichiometric amounts and the reaction initiated with ATP and calcium. Stringent quality control ensures that in the absence of calcium the TT complex completely inhibits myosin ATPase. On addition of 10 µM calcium myosin ATPase will be restored. Calcium binds to Troponin C which dissociates from F-actin allowing myosin to bind.

Reagents

1. Cardiac TT complex (1 x 1 mg, # TT05)
2. Cardiac Myosin S1 (0.25 mg), # MYS03)
3. Cardiac Actin (1 mg, Cat. # AD99-A)
4. ATPase Assay Biochem Kit (Cat. # BK051)
5. 100 mM ATP in 50 mM Tris-HCl pH 7.5 (100ul)
6. 1 M Dithiothreitol in water (100 ul).
7. PM12 Reaction buffer (12 mM Pipes-KOH, pH 7.0, 2 mM $MgCl_2$).
8. 500 mM EGTA-Na pH 8.0.

Equipment

1. Spectrophotometer capable of measuring absorbance at 360 nm (+/- 5 nm bandwidth). We recommend a SpectraMax M2 (Molecular Devices), filter based machines are not suitable.

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2. Half area 96 well microtiter plate (Corning Cat.# 3696 or 3697)
3. Multi-channel pipette

Method
The following major steps are recognized:

Step 1. Assemble required reagents and compounds. (30min).

Step 2. Prepare F-actin polymer stock. (1h).

Step 3. Prepare Thin Filament stock (1.5h)

Step 4. Prepare Motor Mix and plate reader. (15min).

Step 5. Pipette Motor Mix into wells and start reaction/plate reader. (10min).

F-actin polymer stock
1. Resuspend AD99 with 2.5ml of Buffer to 0.4mg/ml (measure protein concentration for better reproducibility), in buffer 5mM Pipes-KOH pH 7.0, 500uM ATP, 500uM DTT.
2. Place at RT for 10min to solubilize the actin.
3. Then add 2.0 mM MgCl2 and 2.0 mM EGTA and incubate at RT for 20 min to polymerize. (shelf life 1h at RT).

Thin Filament stock
1. Resuspend 1 x 1mg TT05 on ice with ice cold water to 5mg/ml. (200ul per vial for 1mg vial).
2. Mix the following to make 1.2 ml of actin/TT05 (TF):
   1000 µl of F-actin stock
   200 µl of TT05
3. Incubate at RT for 20min.
4. Centrifuge at 100K xg 4°C for 1h.
5. Resuspend pellet in 1200 µl of RT PM12 buffer.
6. Store at RT for up to 2h, or 4°C up to 3 days.

Myosin reaction stock
1. Dilute S1 myosin to 1.0 mg/ml with ice cold PM12 buffer.
2. Mix the following in the stated order at RT, to make 2.08 ml of actin/TT/myosin (ATM) control mixture:
   400 µl of PM12
   400 µl 5x MSEG (this is a BK051 component)
   1200 µl of TF
   40 µl of Myosin S1 solution.
   40 µl of 100x PNP (this is a BK051 component)
   10.4 µl of 100mM ATP

3. Using the pre-warmed half area 96-well plate, pipette the following:
4. Pipette 10 µl of 100 µM calcium chloride into “activated” wells.
5. Pipette 10 µl of Milli-Q water into “non-activated” wells.
6. Pipette 10 µl of 10 x [test compound] into appropriate wells.
7. Incubate at 37°C for 2min to warm the mixture.
8. Pipette 100 µl of ATM mixture into all wells.
9. Start protocol, 41 readings, 30 seconds apart, 37°C , OD 360nm.
10. Calculate Vmax and compare non-activated to calcium activated samples.

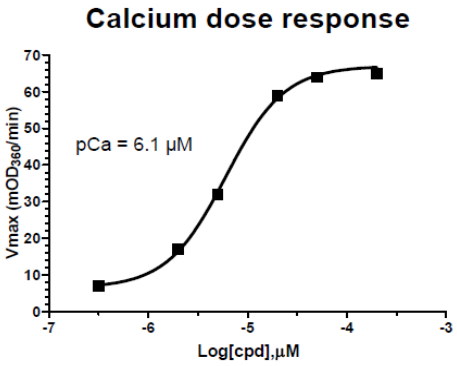

Figure 3 legend: The sarcomere assay was set up as described in the protocol above. Calcium was titrated between 2 and 200 µM and the results plotted on this dose response graph. pCa = 6.1 µM is similar to published pCa values for reconstituted cardiac sarcomeres (Holroyde et al. 1980, Fig.6).

Product Uses
- Measurement of calcium activated myosin ATPase activity when bound to thin filaments.
- Identification/characterization of proteins or small molecules that affect the TT complex regulation and 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.
3. Weeds, A.G., and Taylor, R.S. 1975. Nature (London) 257: 54.
4. M.J. Holroyde et al. 1980. The calcium and magnesium binding sites on cardiac troponin their role in the regulation of myofibrillar adenosine triphosphatase.

Product Citations/Related Products
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