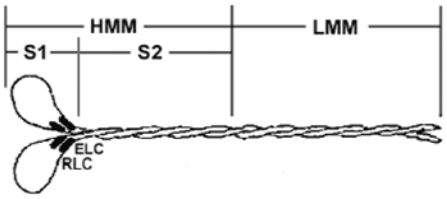
V. 5.0

Myosin Motor Protein (S1 Fragment)
(Bovine Cardiac Muscle)
Cat. # CS-MYS03
250µg
Upon arrival store at 4°C (desiccated)

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 a soluble sarcomere 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 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 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).

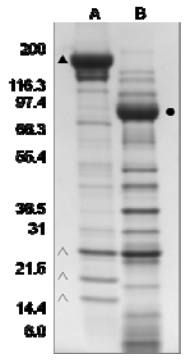
Storage and Reconstitution

Briefly centrifuge to collect the product at the bottom of the tube. Reconstituting a 250 µg tube of MYS03 with 75 µl of Milli-Q water (300 µl for 1 mg size and 1.5 ml for the 5 mg size) 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 purified myosin S1 fragment has been determined to be biologically active during calcium-stimulated ATPase activity using a soluble sarcomere assay. The assay uses a cardiac thin filament complex (CTFC) assembled from purified F-actin (Cat. # AD99) and Tropomyosin/Troponin protein (TT) complex (4). CTFC is composed of six proteins: Actin : Tropomyosin α : Tropomyosin β : Troponin C : Troponin I : Troponin T in a stoichiometric ratio of 7:1:1:1:1:1 (4). These coated filaments are analogous to the thin filaments of muscle fibers (4). Myosin is added in sub-stoichiometric amounts and the reaction initiated with ATP and calcium. Stringent quality control ensures that in the absence of calcium, the CTFC complex 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 Thin Filament Complex (1 x 1 mg; # TFC01)
2. Cardiac Myosin S1 (0.25 mg; # MYS03)
3. ATPase Assay Biochem Kit (Cat. # BK051)
4. 100 mM ATP in 50 mM Tris-HCl pH 7.5 (100 ul)
5. PM12 Reaction buffer (12 mM Pipes-NaOH, pH 6.8, 2 mM $MgCl_2$).

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Equipment

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

Step 2. Prepare Thin Filament stock (15 min).

Step 3. Prepare Motor Mix and plate reader (15 min).

Step 4. Pipette Motor Mix into wells and start reaction/plate reader (10 min).

Thin Filament stock

1. Gently resuspend 1 x 1 mg TFC01 with room temperature (RT) PM12 buffer to 2 mg/ml; it will be a white solution (500 ul per vial for 1 mg vial).
2. Incubate at RT for 10 min.
3. Centrifuge at 500 x g for 30 sec; now it should be a clear solution.
4. Store at RT for up to 20 min.

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 4.0 ml Myosin/Thin Filament control mixture:
   - 2610 µl of PM12
   - 800 µl 5x MSEG (this is a BK051 component)
   - 500 µl of TFC01
   - 30 µl of Myosin S1 solution
   - 40 µl of 100x PNP (this is a BK051 component)
   - 20 µl of 100 mM 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 2 min to warm the mixture.
8. Pipette 100 µl of Myosin/Thin Filament mixture into all wells.
9. Start protocol, 41 readings, 30 seconds apart, 37°C , OD 360 +/- 5 nm.
10. Calculate Vmax and compare non-activated to calcium activated samples.

Figure 3: Calcium Dose Response Curve

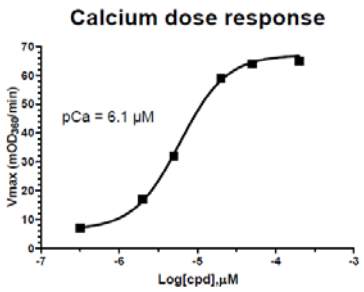

Figure 3 legend: The sarcomere assay was set up as described in the protocol above. Calcium was titrated between 2 and 200 µM and the results plotted on this dose response graph. pCa = 6.1 µM is similar to published pCa values for reconstituted cardiac sarcomeres (ref. 5, Fig. 6).

Product Uses

- Measurement of calcium activated myosin ATPase activity when bound to thin filaments.
- Identification/characterization of proteins or small molecules that affect the CTFC 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. Myosin purification and characterization. *Methods Cell Biol*. 24:333-371.
2. Margossian S.S. and Lowey S. 1982. Preparation of myosin and its subfragments from rabbit skeletal muscle. *Methods Enzymol*. 85:55-71.
3. Weeds A.G. and Taylor R.S. 1975. Separation of subfragment 1 isoenzymes from rabbit skeletal muscle. *Nature*. 257: 54-56.
4. Murray J.M. 1982. Hybridization and reconstitution of the thin filament. *Methods Enzymol*. 85: 15-17.
5. Holroyde M.J., et al. 1980. The calcium and magnesium binding sites on cardiac troponin and their role in the regulation of myofibrillar adenosine triphosphatase. *J. Biol. Chem*. 255: 11688-11693.

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