V. 2.0

Myosin Motor Protein (S1 Fragment)
(Rabbit Skeletal Muscle)
Cat. # CS-MYS04 Lot: 023
Amount: 1 x 250 µg

Upon arrival store at 4°C (desiccated)

Material

Skeletal muscle myosin protein has been purified from rabbit skeletal muscle (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). Rabbit skeletal myosin S1 fragment protein is supplied as a white lyophilized powder.

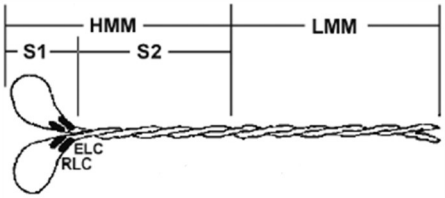


Figure 1. Diagrammatic representation of the myosin protein and its subfragments
Myosin is a hexameric protein consisting of two heavy chains, two essential light chains (ELCs), and two regulatory light chains (RLCs). Myosin can be proteolytically cleaved into heavy meromyosin (HMM) and light meromyosin (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

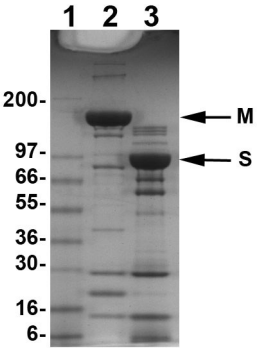
Briefly centrifuge to collect the product at the bottom of the tube. Reconstituting a tube of MYS04 with 50 µl of Milli-Q water will generate a 5.0 mg/ml stock of S1 myosin in the following buffer: 20 mM PIPES pH 7.0, 30 mm KCl, 1 mM EDTA, 5% (w/v) sucrose and 1% (w/v) dextran. The protein should not 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 and its LCs were determined to be 90% pure (see Figure 2).

Figure 2. Full length and S1 myosin.

A 20 µg sample of full length rabbit skeletal myosin protein (lane 2) and the corresponding S1 myosin (lane 3) were separated by electrophoresis using a 4 -20% SDS-PAGE gel and stained with Coomassie Blue. The M indicates the myosin heavy chain (approx. 200 kDa), and S indicates S1 myosin at approx. 90 kDal. Bands at approx. 25 kDa and 14.kDa indicate ELC and RLCs respectively. Protein quantitation was performed using the Precision Red™ Protein Assay Reagent (Cat.# ADV02). SeeBlu molecular weight markers are from Life Technologies Inc.



Biological Activity Assay

The biological activity of rabbit skeletal 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 creating the reaction mix. Myosin is added in sub-stoichiometric amounts to the reaction mix. Stringent quality control ensures that S1 myosin ATPase is at least ten fold higher than S1 myosin alone (see Figure 3). .

Reagents

1. Actin protein (1 mg, # AKL95)
2. Skeletal S1 Myosin (0.25 mg, # CS-MYS04)
3. ATPase Assay Biochem Kit (Cat. # BK051)
4. 100 mM ATP in 50 mM Tris-HCl pH 7.5
5. PM12 Buffer (12 mM Pipes-KOH, pH 7.0, 2 mM $MgCl_2$).
6. 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.
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 chemicals. (30min). Only if screening.
Step 2. Prepare F-actin polymer and myosin stocks. (2h).
Step 3. Prepare Reaction Mix and plate reader. (15min).
Step 4. Pipette Myosin and Reaction Mix into wells and start reaction/plate reader. (10min).

F-actin polymer stock

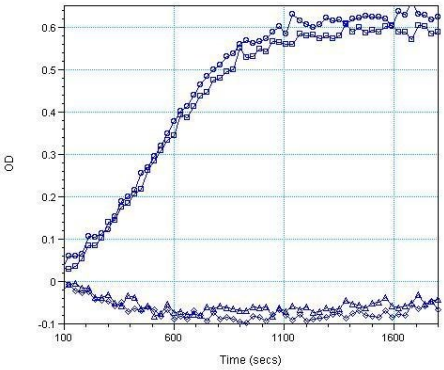
1. Resuspend 1 mg of AKL95, AKL99 or AKF99 with 2.5 ml of Buffer to 0.4 mg/ml, Buffer is 5mM Pipes-KOH, 100 µM ATP, 500 µM DTT.
2. Place at RT for 30 min to depolymerize the actin oligos that form during concentration/lyophilization.
3. Then add 2 mM $MgCl_2$ and 2 mM EGTA and incubate at RT for 1 h to polymerize. (shelf life 1h at RT).

Myosin ATPase assay

1. Dilute S1 myosin to 1 mg/ml with ice cold PM12 Buffer.
2. Resuspend AKL95 to 0.4 mg/ml with 100 µM ATP and 500 µM DTT (2.5 ml per mg).
3. Mix the following to make 1 ml of F-actin reaction mixture:
   700 µl of PM12 Buffer
   300 µl 5x MSEG (from BK051 kit)
   400 µl of F-actin
   12 µl of 100x PNP (from BK051 kit)
   6 µl of 100 mM ATP

4. Using the pre-warmed half area 96-well plate, pipette the following:
5. Pipette 2 µl of myosin into wells B1,2.
6. Pipette 130 µl of F-actin reaction mix into wells A1,2 and B1,2.
7. Start protocol, 41 readings, 30 seconds apart, 37°C , OD 360nm.
8. Calculate Vmax and compare non-activated to calcium activated samples.
9. Note: For compound screening myosin can be mixed with the reaction mix and pipette onto compound solution in the well. Compound solution can be 50x or 100x strength in DMSO.

Figure 3. ATPase assay of MYS04



Legend: Representative data for the ATPase assay described in the Biological Activity Assay section in the presence (circles and squares) or absence (triangles and diamonds) of 15 µg/ml MYS04.

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.
3. Weeds, A.G., and Taylor, R.S. 1975. Nature (London) 257: 54.

Product Citations/Related Products

For the latest citations and related products please visit www.cytoskeleton.com.