V. 1.0

# Myosin Motor Protein (S1 Fragment) (Rabbit Psoas Muscle)

**Cat. # CS-MYS04**

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

## Material

Skeletal muscle myosin protein has been purified from rabbit psoas 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 psoas myosin S1 fragment protein is supplied as a white lyophilized powder.

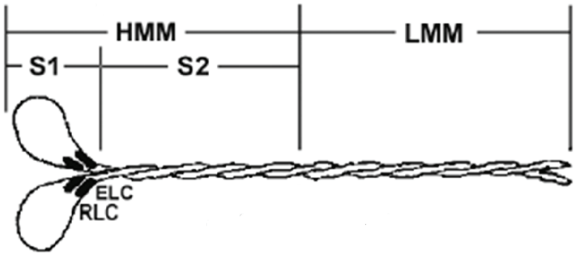



**Figure 1. Diagrammatic representation of the myosin protein and its subfragments**
Myosin is a hexameric protein consisting of two heavy chains and two light chains. Myosin can be proteolytically cleaved into heavy meromyosin (HMM) and light meromyosin (LMM) by α-chymotrypsin in the presence of magnesium. In the presence of EDTA, however, α-chymotrypsin produces the soluble myosin S1 fragment (3).

## Storage and Reconstitution

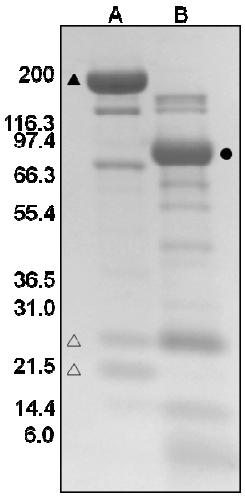
Briefly centrifuge to collect the product at the bottom of the tube. Reconstituting a tube of MYS04 with 75 µl of Milli-Q water will generate a 3.3 mg/ml stock of psoas S1 myosin in the following buffer: 20 mM PIPES pH 7.0, 1 mM EDTA, 5% (w/v) sucrose and 1% (w/v) dextran. The protein should not be exposed 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 was determined to be 90% pure (see Figure 2).

**Figure 2. Full length and S1 myosin.**

A 20 µg sample of full length rabbit psoas 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 closed triangle indicates the myosin heavy chain (approx. 200 kDa), open triangles indicate the RLC (approx. 20 kDa) and two ELC isoforms (approx. 25 and 21 kDa), and the S1 fragment (approx. 97 kDa) is indicated with a closed circle. 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 rabbit psoas 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. Upon addition of 2 mM calcium, myosin ATPase will be restored (see Figure 3). Calcium binds to Troponin C which dissociates from F-actin allowing myosin to bind.

## Reagents

1. Cardiac TT complex (0.5 mg, # CS-TT05)
2. Psoas S1 Myosin (1 mg, # CS-MYS04)
3. Cardiac Actin (1 mg, # CS-ADMK)
4. ATPase Assay Biochem Kit (Cat. # BK051)
5. 100 mM ATP in 50 mM Tris-HCl pH 7.5
6. PM12 Buffer (12 mM Pipes-KOH, pH 7.0, 2 mM $MgCl_2$).
7. 500 mM EGTA-Na, pH 8.0.


**Phone:** (303) 322.2254 **Fax:** (303) 322.2257
**Customer Service:** cserve@cytoskeleton.com
**Technical Support:** tservice@cytoskeleton.com

**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 stock. (2h).
Step 3. Prepare Motor Mix and plate reader. (15min).
Step 4. Pipette Motor Mix into wells and start reaction/plate reader. (10min).

**F-actin polymer stock**

1. Resuspend ADMK or AD99 with 2.5 ml of Buffer to 0.4 mg/ml, Buffer is 5mM Pipes-KOH, 100 uM ATP, 500uM 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 TT05 on ice with ice cold water to 5mg/ml. (100 µl per vial for 0.5 mg vial).
3. Mix the following to make 1 ml of actin/TT/myosin (ATM) control mixture:
   150 µl of F-actin (0.4 mg/ml in 5mM Pipes pH 7.5, 2 mM MgCl2, 58 µM $CaCl_2$, 2 mM EGTA, 100 µM ATP, 500 µM DTT)
   100 µl of TT05
   80 µl of PM12 Buffer
   100 µl 5x MSEG
   5 µl of 100x PNP
4. Wait for 10 min to make thin filaments.
5. Add 2.5 µl of S1 myosin and 5 ul of 50 mM ATP and mix.
6. Incubate at 37°C for 3 min.
7. Using the pre-warmed half area 96-well plate, pipette the following:
8. Pipette 10 µl of 2 mM calcium into “activated” wells.
9. Pipette 10 µl of Milli-Q water into “non-activated” wells.
11. Pipette 90 µl of myosin/TT/actin mixture into all wells.
12. Start protocol, 41 readings, 30 seconds apart, 37°C , OD 360nm.
13. Calculate Vmax and compare non-activated to calcium activated samples.

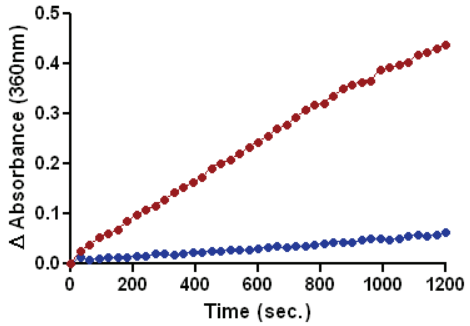



**Figure 3. Absorbance traces for the ATPase assay of MYS04**
Representative data for the ATPase assay described in the Biological Activity Assay section using 2.5 µg/ml MYS04 in the presence (red circles) or absence (blue circles) of 200 µM $CaCl_2$.

**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.