V. 1.0

Myosin Motor Protein (S1 Fragment)
(Chicken gizzard)
Cat. # CS-MYS05

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

Material

Smooth muscle myosin protein has been purified from chicken gizzards (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 papain to liberate the soluble subfragment-1 (S1) domain, which was isolated by centrifugation (3, 4). The purified myosin S1 fragment has been determined to be biologically active in an F-actin activated ATPase assay (see biological activity assay). Chicken gizzard S1 myosin protein is supplied as a white lyophilized powder.

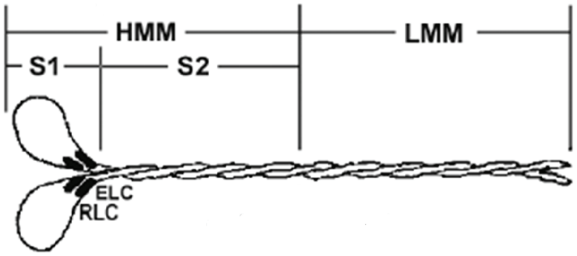


Figure 1. Diagrammatic representation of the myosin protein and its subfragments
Myosin is a hexameric protein consisting of two heavy chains and two light chains. Myosin can be proteolytically cleaved into heavy meromyosin (HMM) and light meromyosin (LMM) by α-chymotrypsin and cleavage by papain produces soluble S1 myosin and the insoluble myosin tail (3, 4)

Storage and Reconstitution

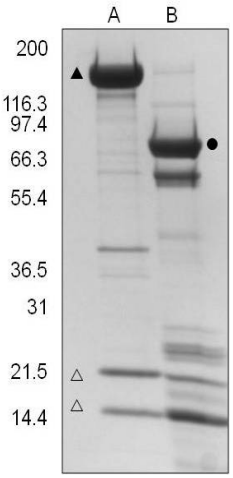
Briefly centrifuge to collect the product at the bottom of the tube. Reconstituting a tube of CS-MYS05 with 180 µl of Milli-Q water will generate a 1.4 mg/ml stock of gizzard S1 myosin in the following buffer: 8 mM PIPES pH 7.0, 8 mM $MgCl_2$, 5% (w/v) sucrose and 1% (w/v) dextran. The protein should 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 Chicken gizzard 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 light chains (approx. 17 and 20 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 chicken gizzard S1 myosin can be determined from its rate of F-actin activated ATP hydrolysis. The assay is constructed by first polymerizing actin to form F-actin. Myosin is then added in substoichiometric amounts and the reaction is initiated with ATP.

Reagents

1. Gizzard S1 Myosin (0.25 mg, # CS-MYS05)
2. Cardiac Actin (1 mg, # CS-ADMK)
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 (e.g. Corning Cat.# 3697)
3. Multi-channel pipette

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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 0.5 ml of Buffer to 2 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 0.5 mg/ml with ice cold PM12 Buffer containing 1mM DTT.
2. Mix the following to make 0.49 ml of actin/myosin mixture:

   250 µl of F-actin (2 mg/ml in 5mM Pipes pH 7.5, 2 mM MgCl2, 58 µM $CaCl_2$, 2 mM EGTA, 100 µM ATP, 500 µM DTT). Replaced with equal volume PM12 Buffer for no F-actin control.
   120 µl of PM12 Buffer
   100 µl 5x MSEG
   5 µl of 100x PNP
   10 ul of S1 myosin (0.5 mg/ml)

3. Add 5 ul of 50 mM ATP and mix.
4. Incubate at 37°C for 3 min.
5. Using the pre-warmed half area 96-well plate, pipette the following:
6. Pipette 10 µl of Milli-Q water into each well.
7. Pipette 90 µl of actin/myosin mixture per well.
8. Start protocol, 41 readings, 30 seconds apart, 37°C , OD 360nm.
9. Calculate Vmax and compare non-activated to F-actin activated samples.

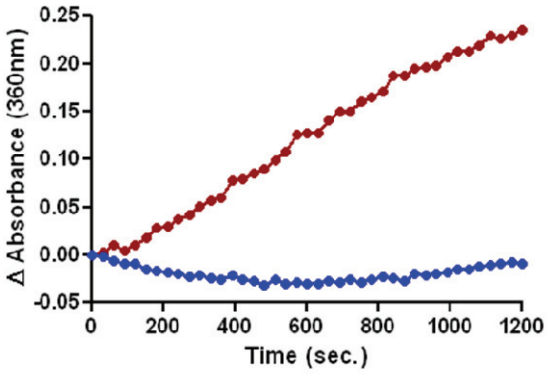

Figure 3. Absorbance traces for the ATPase assay of MYS05
Representative data for the ATPase assay described in the Biological Activity Assay section using 10 µg/ml CS-MYS05 in the presence (red circles) or absence (blue circles) of 1mg/ml F-actin.

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. Persechini, A., and Hartshorne, D.J. 1983. Biochemistry 22: 470-476
2. Trybus, K.M. 1994. J. Biol. Chem. 269:20819-20822.
3. Greene, L.E. *et al.* 1983. Biochemistry 22, 530-535.
4. Seidel, J.C. 1980. J. Biol. Chem. 255, 4355-4361.

Product Citations/Related Products

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