V. 1.2

Heavy Meromyosin Protein (HMM Fragment)
(Bovine Cardiac Muscle)
Cat. # CS-MH03 Lot 033
Spec. Act. approx. 110 nmoles/min/mg

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

Material

Cardiac myosin protein has been purified from bovine heart tissue (1,2,3). 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 in the presence of $MgCl_2$ to liberate the soluble heavy fragment (HMM) domain, which was isolated by centrifugation followed by anionic exchange chromatography to remove S1 (2,3). The purified myosin HMM 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.

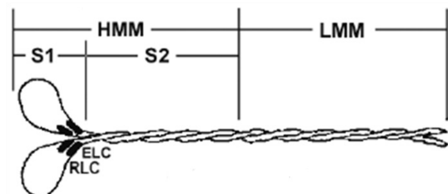


Figure 1. Diagrammatic representation of the myosin protein and its subfragments

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 (2,3).

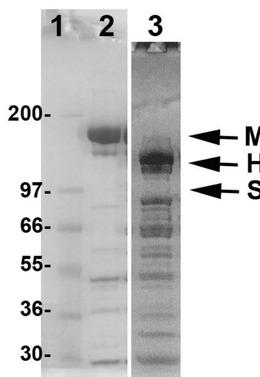
Storage and Reconstitution

Briefly centrifuge to collect the product at the bottom of the tube. Reconstituting a 100 µg tube of MH03 with 100 µl of 10 mM Tris-HCl pH 7.50, 30 mM KCl, 1 mM EDTA, 1 mM DTT in Milli-Q water The protein should not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable at 4°C desiccated (<10% humidity) for 6 months.

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). After chymotrypsin digestion and FPLC the HMM and light chains constitute approx. 85% of total protein. The S1 myosin content is less than 1%.

Figure 2. Full length and HMM myosin.



A sample of full length bovine cardiac myosin protein (20 µg lane A) and the corresponding HMM myosin (40 µg lane B) were separated by electrophoresis using a 4-20% SDS-PAGE gel and stained with Coomassie Blue. The M indicates the myosin heavy chain (approx. 240 kDa), whereas H indicates HMM and S indicates S1 myosin. Protein quantitation was performed using the Precision Red™ Protein Assay Reagent (Cat.# ADV02). SeeBlue molecular weight markers are from Life Technologies.

Biological Activity Assay

The biological activity of bovine cardiac myosin HM 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, HMM is added in substoichiometric amounts and the reaction initiated with ATP. Stringent quality control ensures that F-actin stimulated HMM ATPase is over two fold that of HMM alone and that the specific activity is in line with published values of 25-100 nmoles/min/mg or 0.5 to 2.0 ATPs/head/s. ATPase activity is also stimulated by addition of actin thin filaments (Cat:# TFC01) and 10 µM calcium. Calcium binds to Troponin C which dissociates from F-actin allowing myosin to bind.

Reagents

1. Cardiac Heavy Meromyosin HMM (0.10 mg), # MH03)
2. Cardiac Actin (1 mg, Cat. # AD99-A)
3. ATPase Assay Biochem Kit (Cat. # BK051)
4. 100 mM ATP in 50 mM Tris-HCl pH 7.5 (100ul)
5. 1 M Dithiothreitol in water (100 ul).
6. PM12 Reaction buffer (12 mM Pipes-KOH, pH 7.0, 2 mM $MgCl_2$).
7. 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

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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 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 1 mg AD99 or AKL99 with 1.0 ml of 1 mM DTT to create 1.0 mg/ml F-actin (measure protein concentration for better reproducibility).
2. Place at RT for 10 min to solubilize the actin.
3. Then add 2 mM $MgCl_2$ and 2.0 mM EGTA and incubate at RT for 20 min to polymerize. (shelf life 2h at RT).

Myosin reaction stock
1. Dissolve 100 µg HMM to 1.0 mg/ml with 100 µl ice cold PM12 buffer and place on ice.
2. Make control Buffer: 10 mM Pipes-NaOH pH 7.0 plus 2 mM $MgCl_2$, 1 mM DTT, 2.0 mM EGTA and 20 µM $CaCl_2$ (if present in the actin stock as it is in AD99 and AKL99).
3. Mix the following (µl) in a half area well plate in the stated order 1>2>3 at RT.

Well ID.	1. Buffer	2. F-Actin	3. HMM
A1,2 Buffer	25	--	--
B1,2 F-actin	2.0	23	--
C1,2 HMM	23	--	2.0
D1,2 HMM + F-actin	--	23	2.0

4. Dilute 10 µl of 100 mM ATP with 90 µl Milli-Q water and pipette 5 µl of this into each well.
5. Place the plate at 37°C for 30 min.
6. Add 120 µl of Cytophos Reagent to each well at RT for 10min, and read at 650 nm to detect liberated phosphate.

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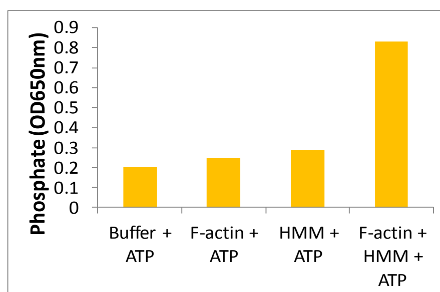

Figure 3 legend: F-actin stimulated HMM ATPase. Reactions contained control buffer plus 1.5 mM ATP. F-actin was added to 0.5 mg/ml, and HMM to 66 ug/ml. After 30 min at 37°C reactions were quenched by adding 120 ul of Cytophos Reagent. Signals developed quickly and the Cytophos reaction reach maximum signal after 3-4min instead of the usual 10min, the signals were read at 650 nm to measure free phosphate. In this scenario an OD of 0.60 is equivalent to approximately 7.2 nmoles of phosphate. Which calculates to a specific activity of 120 nmoles/min/mg.

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Product Uses
- Measurement of calcium activated cardiac HMM ATPase activity when bound to thin filaments.
- Identification/characterization of proteins or small molecules that affect the TT complex regulation and cardiac HMM ATPase activity
- Identification/characterization of proteins or small molecules that affect cardiac HMM / 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. Margossian, S.S.. 1985. JBC, 260 (25), 13747-13754.
4. Weeds, A.G., and Taylor, R.S. 1975. Nature (London) 257: 54.
5. 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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