Cardiac Thin Filament Complex (Bovine Cardiac Muscle)  
# CS-TFC01 Lot 014

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

Material  
The cardiac thin filament complex (CTFC) has been assembled from purified F-actin (Cat. # AD99) and Tropomyosin / Troponin protein (TT) complex (Cat. # TT05). Thus, 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; see Figure 1. After assembly, the thin filaments are centrifuged at 100,000 x g and the pellets resuspended in reaction buffer which purifies the calcium sensitive complex. The complex has been determined to be biologically active in a calcium activated myosin ATPase assay (see biological activity assay). The complex is supplied as a white lyophilized powder.

Storage and Reconstitution  
Briefly centrifuge to collect the product to the bottom of the tube. The protein should be reconstituted to 2 mg/ml by the addition of room temperature (RT) PM12 buffer. Do not pipette vigorously as this will denature the filaments. A white solution will appear first. Leave this for 10 min, then centrifuge at 500 x g to remove air bubbles. The solution should now look clear. Do not centrifuge at high speed because the filaments will sediment.

The protein will be in the following buffer: 32 mM PIPES pH 6.8, 25 mM KCl, 2 mM MgCl₂, 5% (w/v) sucrose, and 1% (w/v) dextran.

The protein solution can be frozen by aliquoting into "experiment sized" amounts, snap frozen in liquid nitrogen and stored at -70°C. The protein is stable for 6 months if stored at -70°C. 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 purity of the CTFC is >95% (Figure 2, lane 2).

Figure 1—Schematic diagram of muscle thin filament.

Figure 2. Thin filament protein analysis.

Legend: A 20 µg sample of CTFC proteins was separated by electrophoresis in a 4-20% SDS-PAGE system (Lane 2) and stained with Coomassie Blue. Molecular weights are 43 kDa actin, 38 kDa Troponin T, 32 kDa Tropomyosin (α+β), 24 kDa Troponin I, and 18 kDa Troponin C. Protein concentration was measured with the Precision Red™ Protein Assay Reagent (Cat.# ADV02). SeeBlue molecular weight markers (Lane 1) are from Life Technologies Inc.

Biological Activity Assay  
The biological activity of the CTFC can be determined from its ability to regulate myosin ATPase activity. The CTFC re-creates coated filaments which are analogous to the thin filaments of muscle fibers. Myosin is added in substoichiometric amounts and the reaction is initiated with ATP and calcium. Stringent quality control ensures that in the absence of exogenous calcium, the CTFC completely 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, # MY503)  
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 MgCl₂).

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...
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 temp 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 30s; now it is a clear solution.
4. Store at room temperature 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 of 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
   20 µl of 100 mM ATP
   40 µl of 100x PNP (this is a BK051 component)
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 [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.

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 (Holroyde et al. 1980, 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 regulation and myosin ATPase activity
- Identification/characterization of proteins or small molecules that affect myosin/F-actin interaction

References

Product Citations/Related Products
For the latest citations and related products please visit www.cytoskeleton.com.