V. 4.1

Alpha-Actinin
Source: Rabbit skeletal muscle
Cat. # AT01

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

Background Information

Alpha-actinin is an actin filament (F-actin) binding and crosslinking protein (1). It is found in skeletal muscle, smooth muscle and non-muscle cells; four distinct isotypes, ACTN1-ACTN4 are known in humans (2,3). The isotypes have distinct calcium sensitivities toward binding F-actin. Alpha-actinins from skeletal muscle sources (ACTN-2 & ACTN-3) are not sensitive to calcium while ACTN-1 & ACTN-4 contain calcium sensitive EF hands (4).

Alpha-actinin isotypes range in size from 95 to 115 kDa. Their general structure is similar as they have an amino-terminal actin binding domain, a central section containing four spectrin-like repeats and a carboxy terminal containing the calcium binding motifs and EF-hand structures. The central section is thought to enable dimerization in anti-parallel fashion, thus connecting the calcium binding domain of one monomer with the actin binding domain of the other (5).

Alpha-actinin immunolocalizes to adherens-type junctions and extracellular matrix junction (6). The protein appears to play a role in the transduction of integrin mediated signals from the extracellular matrix to the internal cytoskeleton (7). Also, evidence suggests that another function is to crosslink microfilaments in the cytosol, thus contributing to the gel/sol phase in the cytoplasm (8).

Material

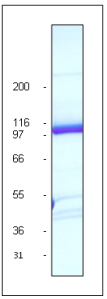
Alpha-actinin is purified from rabbit skeletal muscle. Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gel. Purity is >85% (Figure 1).

Alpha-actinin runs as approximately a 100 kDa band on SDS-PAGE (Figure 1). It is supplied as a white lyophilized powder.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized protein can be stored desiccated to <10% humidity at 4°C for 1 year. For reconstitution, briefly centrifuge to collect the product at the bottom of the tube and resuspend to 1 mg/ml with Milli-Q water. The protein will then be in the following buffer: 4 mM Tris-HCl pH 7.6, 4 mM NaCl, 20 µM EDTA, 1% (w/v) sucrose, and 0.2% (w/v) dextran. The concentrated protein should be aliquoted into experiment sized amounts, snap frozen in liquid nitrogen and stored at –70°C where it is stable for 1 year. Note: Avoid repeated freeze-thaw cycles.

Figure 1: α-actinin Protein Purity Determination



Legend: 10 µg of α-actinin protein was separated by electrophoresis in a 4-20% SDS-PAGE system. The protein was stained with Coomassie Blue. Protein quantitation was determined with the Precision Red™ Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen. Alpha-actinin runs at approximately 100 kDa.

Biological Activity

The biological activity of alpha-actinin is assessed by its ability to bind to F-actin filaments. An F-actin binding assay is performed. Under the assay conditions described below >65% of alpha-actinin co-pellets with F-actin while <10% of alpha-actinin pellets in the absence of F-actin (Figure 2).

Co-sedimentation assay Method

1) F-actin (16.8 µM final concentration) is mixed with alpha-actinin (2 µM final concentration) in the following buffer: 5 mM Tris pH 7.0, 2 mM magnesium chloride, 0.2 mM calcium chloride, 50 mM potassium chloride, 1 mM ATP, 5 mM guanidine carbonate. NOTE: guanidine carbonate is a component of the ATP stock and is not required for the co-sedimentation assay.
2) Control assays of F-actin only and alpha-actinin only are included. Bovine serum albumin is included as a non-actin binding protein control.
3) The reactions are incubated for 30 minutes at room temperature and then centrifuged at 100,000 x g for 30 minutes at room temperature. Supernatant and pellet fractions are analysed by SDS-PAGE and proteins are visualized by Coomassie Blue staining (Figure 2).

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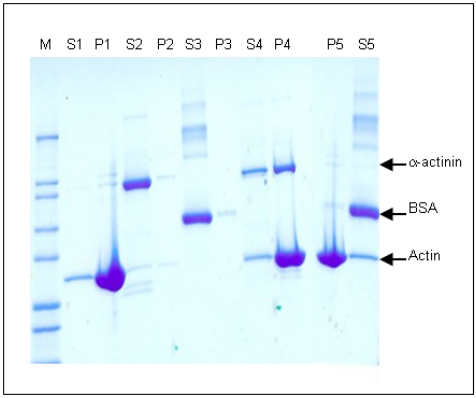
Figure 2. F-actin Binding Assay



Legend: F-actin binding assay was performed as described in Biological Assay Method. S1, supernatant F-actin alone; P1, pellet F-actin alone; S2, supernatant alpha-actinin alone; P2, pellet alpha–actinin alone; S3, supernatant BSA alone; P3, pellet BSA alone; S4, supernatant F-actin/alpha-actinin; P4, pellet F-actin/alpha-actinin; S5, supernatant F-actin/BSA; P5, pellet F-actin/BSA. It can be seen that alpha actinin will only pellet in the presence of F-actin (compare S2/P2 and S4/P4).

Product Uses

- Biochemical analysis of alpha-actinin functions
- Study the regulation of actin dynamics
- Positive control of actin binding assays
- Positive control for actin bundling assays

References

1. Burridge and Feramisco, 1981. *Nature.* **294**: 565-567
2. Beggs et al., 1992. *J. Biol. Chem*. **267**: 9281-9288
3. Berman, et al., 2010. *Physiol*. **25**: 250-259
4. Burridge, 1981. *Science.* **294**: 565-567
5. Fukami et al., 1994. *J. Biol. Chem*. **269**: 1518-1522
6. Noegel et al., 1987. *FEBS Lett*. **221**: 391-96
7. Sanger et al., 1983. *J. Cell Biol*. **96**: 961-69
8. Otey et al., 1990. *J. Cell Biol*. **111**: 721-729
9. Pacaud and Harricane, 1993. *Biochem*. **32**: 363-374

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