V. 1.0

**Arp2/3 Protein Complex**
**Source: Bovine brain**
**Cat. # RP01**

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

## Material

The Arp2/3 complex (Actin-Related Proteins) has been purified from bovine brain and consists of seven evolutionarily conserved protein subunits (1), all present in approximately equal stoichiometry (see Figure 1). The Arp2/3 complex (molecular weight of 224 kDa) is a key regulator of branched actin filament nucleation and is important for cell locomotion, phagocytosis and intracellular motility. Arp2/3 complex is supplied as a white lyophilized powder.

## Storage and Reconstitution

Store lyophilized product desiccated (<10% humidity) at 4°C where it is stable for 6 months. Resuspend the protein complex to 5 mg/ml with 10 µl of cold Milli-Q water. When resuspended the complex is in the following buffer: 20 mM Tris pH 7.5, 25 mM KCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.1 mM ATP, 1.0% (v/v) dextran and 5% (v/v) sucrose. The protein should then be aliquoted into experiment sized amounts, snap frozen in liquid nitrogen and stored at -70°C where it is stable for 6 months. Avoid multiple freeze-thaw cycles. Further dilution of Arp2/3 should be made in the following buffer: 20 mM Tris pH 7.5, 25 mM KCl, 1 mM $MgCl_2$ and 1 mM DTT (Note: add DTT to the buffer immediately prior to use).

## Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gel. Arp2/3 protein complex is determined to be 90% pure (see Figure 1).

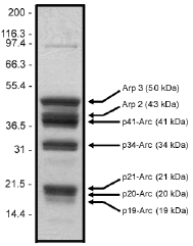


**Figure 1. Arp2/3 Protein Purity Determination.** A 10 µg sample of Arp2/3 complex was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined with the Precision Red™ Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

## Biological Activity Assay

The Arp2/3 complex is able to induce the branched polymerization of actin filaments *in vitro* at a molar ratio of 1:200 (Arp2/3:actin). This stimulation is observable in an *in vitro* polymerization assay (described below), however, the stimulation from Arp2/3 alone is very low under these conditions. In the presence of N-WASP protein (or the VCA domain of N-WASP, Cat. # VCG03) the nucleating activity of Arp2/3 is greatly enhanced. The *in vitro* polymerization assay is described below:

## Reagents

1. Arp2/3 protein complex (Cat. # RP01)
2. VCA domain-GST fusion (Cat # VCG03)
3. Pyrene labeled actin (Cat. # AP05)
4. Polymerization buffer 1.5x stock: 7.5 mM Tris pH 7.5, 75 mM KCl, 3 mM $MgCl_2$, 1.5 mM EGTA, 0.15 mM CaCl, 0.75 mM DTT, 0.3 mM ATP [add fresh from a 100 mM stock pH 7.0, immediately prior to use].
5. General Actin Buffer (Cat. # BSA01):10 mM Tris pH 7.5, 0.2 mM $CaCl_2$, 0.2 mM ATP, 1 mM DTT.

## Equipment

1. Fluorescence spectrophotometer with an excitation wavelength of 360 +/- 20 nm and an emission wavelength of 405 +/- 10 nm or 420 +/- 20 nm. Cytoskeleton recommends the SPECTRAFluor Plus from TECAN Austria GmbH or Gemini from Molecular Devices Inc.
2. Black polystrene 96 well assay plate (Costar, Cat. # 3915).

## Method

1. Resuspend and dilute pyrene labeled muscle actin (Cat. # AP05) to 0.45 mg/ml (10.4 µM) with General Actin Buffer (Cat. # BSA01) supplemented with 0.2 mM ATP and 1 mM DTT. Leave on ice for 1 h to depolymerize actin oligomers.
2. Centrifuge the actin at 14,000 rpm at 4°C for 30 min to remove residual nucleating centers.
3. Pipette the top 80% of the supernatant into a new microfuge tube on ice.
4. Dilute the Arp2/3 complex (Cat. # RP01) to 0.3 mg/ml in G-buffer. Keep on ice.
5. Resuspend one tube of VCA domain protein (Cat. # VCG03) to 1 mg/ml by adding 500 µl of Milli-Q water. Pipet up and down slowly to resuspend the pellet. Keep on ice.
6. Just before use, dilute the pyrene-labeled actin to 0.1 mg/ml in ice-cold General Actin Buffer.


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7. Add the following components to the 96 well assay plate:

| Well | Poly buffer (µl) | RP01 (µl) | VCA domain (µl) |
|---|---|---|---|
| A1 | 200 | 0 | 0 |
| B1 | 200 | 0 | 0 |
| C1 | 200 | 0 | 5 |
| D1 | 200 | 0 | 5 |
| E1 | 200 | 2 | 0 |
| F1 | 200 | 2 | 0 |
| G1 | 200 | 2 | 5 |
| H1 | 200 | 2 | 5 |

8. Using a multi-channel pipet, add 100 µl of diluted pyrene-labeled actin to wells A1-H1 of the assay plate. Note: Do not introduce air bubbles into the wells.
9. Place the 96 well plate into the fluorescent spectrophotometer and read the samples for 1 h.
10. In the assay described above, pyrene actin is present at a final concentration of 0.8 µM, Arp2/3 complex at 10 nM and VCA domain at 400 nM.
11. Results for a typical actin polymerization assay is shown in Figure 2.

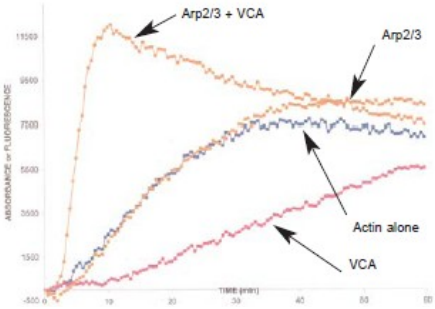


**Figure 2. Actin Polymerization Assay with Arp2/3 and VCA domain proteins.** Actin polymerization was carried out as described in the method; all reactions contain pyrene labeled actin. Reactions containing Arp2/3 or VCA in addition to actin show little or no enhancement of actin nucleation. In the presence of the VCA domain however, Arp2/3 results in an enhancement of actin nucleation (Note the steep nucleation phase of polymerization). Actin polymerization is measured in arbitrary fluorescent units over time.

**Product Uses**

- Characterization of actin polymerization factors
- Formation of branched actin filaments
- Antibody standard for Western blot analysis

**References**

1. Pollard, T.D. et al. 2000. Ann. Rev. Biophys. Biomol. Struct. 29: 545-576.

**Product Citations/Related Products**

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