V. 2.0

**Arp2/3 Protein Complex**
**Source: Porcine brain**
**Cat. # RP01P**

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

## Background Information

Arp2/3 complex is a seven-subunit protein that plays a major role in the regulation of the actin cytoskeleton and is found in most in eukaryotic cells [1]. Two of its subunits, the Actin-Related Proteins ARP2 and ARP3 closely resemble the structure of monomeric actin and serve as nucleation sites for new actin filaments. The complex binds to the sides of existing ("mother") filaments and initiates growth of a new ("daughter") filament at a distinctive 70 degree angle from the mother. Branched actin networks are created as a result of this nucleation of new filaments. The Arp2/3 complex (molecular weight of 224 kDa) is a key regulator of branched actin filament nucleation and is important for cell locomation, phagocytosis and intracellular motility.

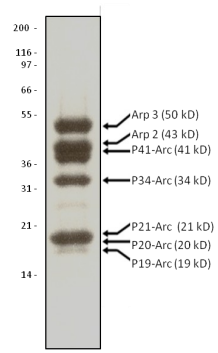
## Material

The Arp2/3 complex (Actin-Related Proteins) has been purified from porcine 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 locomation, phagocytosis and intracellular motility. Arp2/3 complex is supplied as a white lyophilized powder.

RP01P from procine brain replaces RP01 from bovine brain. We have compared the activity of Arp2/3 complex from both sources and have found them to behave identically in actin polymerization assays (see Fig. 2)

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**




Legend: 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 Life Technologies.

## 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).

## 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. # RP01P)
2. Rabbit Muscle Actin Protein, >95% Pure (Cat# AKL95)
3. VCA domain-GST fusion (Cat # VCG03)
4. Pyrene labeled actin (Cat. # AP05)
5. General Actin Buffer (Cat. # BSA01):10 mM Tris pH 7.5, 0.2 mM $CaCl_2$, 0.2 mM ATP, 1 mM DTT.
6. 10X Actin Polymerization Buffer (Cat# BSA02): 20 mM $MgCl_2$, 500 mM KCl, 10 mM ATP, 0.05M Guanidine Carbonate pH 7.5

## 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).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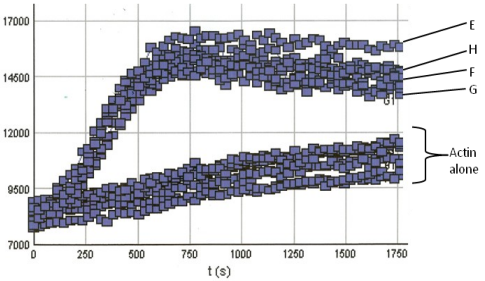
**Method**

1. Resuspend and dilute pyrene labeled muscle actin (Cat. # AP05) and rabbit muscle actin (Cat# AKL95) to 0.2 mg/ml each with water supplemented with 0.2 mM ATP and 1 mM DTT. Leave at room temperature for 1 h to depolymerize actin oligomers.
2. Mix equal volumes of AP05 and AKL95 in sufficient quantity to cover the number of assays being performed (100 µl per assay). This is your actin stock.
3. Centrifuge the actin stock at 30,000 rpm (100,000 x g) for 30 minutes at 4°C to remove residual nucleating centers. NOTE: The ultracentrifugation step gives optimal actin protein for polymerization assays, however, if an ultracentrifuge is not available the a 30 minute centrifugation at 14,000 rpm ( 20,000 x g) at 4°C will help to get rid of protein aggregates that will interfere with actin polymerization.
4. Pipette the top 80% of the supernatant into a microfuge tube. Dilute the actin stock 1:1 with water plus 0.2 mM ATP/1 mM DTT.
5. Dilute the Arp2/3 complex (Cat. # RP01P) to 0.3 mg/ml in Milli-Q water. Keep this stock on ice.
8. Resuspend one tube of VCA domain protein (Cat. # VCG03) to 0.125 mg/ml in Milli-Q water. Pipet up and down slowly to resuspend the pellet. Keep this stock on ice.
9. Carry out polymerizations in a black 96 well plate (300 µl volume well). Table 1 shows some typical reaction conditions and control reaction conditions for Arp2/3 stimulation of actin polymerization.

| Well | 1.5X Polymerization buffer (µl) | RP01P Stock (µl) | VCA domain Stock (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 |

10. Place the plate in the fluorescent spectrophotometer and allow to incubate at room temperature (25 °C) for 15 minutes.
11. Using a multi-channel pipet, add 100 µl of the diluted actin stock to reaction wells. Note: Do not introduce air bubbles into the wells.
11. Place the 96 well plate into the fluorescent spectrophotometer and read the samples for 30 min.
12. In the assay described above, pyrene actin and rabbit muscle actin are present at a final concentration of 0.8 µM, Arp2/3 complex at 10 nM and VCA domain at 400 nM.
13. Results for a typical actin polymerization assay is shown in Figure 2. NOTE: the VCA alone and Arp2/3 alone results are not shown in Fig.2 as they show no stimulation of actin polymerization. Fig. 2 shows a comparison between bovine and porcine derived Arp2/3 complex.

**Figure 2. Actin Polymerization Assay with Arp2/3 and VCA domain proteins.**




**Legend:** Actin polymerization was carried out as described in the method; All reactions contain 0.8 µM actin. Reactions containing Arp2/3 or VCA (data not shown) show little or no enhancement of actin nucleation. Actin alone also shows low polymerization competence under these conditions (Actin alone reactions). 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. In our tests porcine derived Arp2/3 (wells E & F) behaved identically to Arp2/3 derived from bovine sources (wells G & H).

**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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