

Actin and Actin Conjugates

Quick Facts

Storage upon receipt:

- $\leq -20^{\circ}\text{C}$ for unlabeled actin (A-12375)
- $\leq -70^{\circ}\text{C}$ for labeled actin (A-12373, A-12374, A-34050, A-34051)
- Avoid freeze-thaw cycles

Introduction

Unlabeled and fluorescently labeled actin have become important tools for investigating cytoskeleton dynamics *in vivo*.¹⁻⁵ Molecular Probes offers unlabeled actin from rabbit muscle, as well as fluorescently labeled actin conjugates prepared with our proprietary Alexa Fluor® dyes (Table 1). These superior dyes exhibit high absorbance at wavelengths of maximal output of common excitation sources, bright and photostable fluorescence and insensitivity to pH over a broad range.

All of our fluorescently labeled actin conjugates are prepared by reacting amine residues of polymerized actin with the succinimidyl esters of the appropriate dye using a modification of the method described by Alberts and coworkers.² After labeling, the conjugates are subjected to depolymerization and subsequent polymerization to ensure that the actin conjugates are able to assemble properly. The polymerized labeled actin is then separated from remaining monomeric actin by centrifugation, depolymerized and packaged in monomeric form.

Materials

Contents

Unlabeled actin is supplied in a 1 mg unit size lyophilized from 1 mL of 2 mM Tris, pH 8.0, 0.2 mM ATP. Fluorescently labeled actin conjugates are supplied in solution in unit sizes of 200 μg of conjugate at 3–5 mg/mL in G buffer (5 mM Tris, pH 8.1, 0.2 mM CaCl_2 , 0.2 mM dithiothreitol, 0.2 mM ATP) containing 10% (w/v) sucrose. The exact concentration of the protein is indicated on the product's label.

Table 1. Actin conjugates.

Cat #	Label	Abs *	Em *
A-12373	Alexa Fluor 488	495	519
A-12374	Alexa Fluor 568	578	603
A-34050	Alexa Fluor 594	590	617
A-34051	Alexa Fluor 647	650	668

* Absorption (Abs) and fluorescence emission (Em) maxima, in nm.

Storage and Handling

Upon receipt, unlabeled actin should be stored desiccated at $\leq -20^{\circ}\text{C}$. Lyophilized actin is stable for at least 6 months under these conditions. A 2 mg/mL stock solution of unlabeled actin can be prepared by adding 0.5 mL of 2 mM Tris, pH 8.0, directly to the vial. The reconstituted actin should be aliquotted in small volumes and stored at $\leq -70^{\circ}\text{C}$. Reconstituted unlabeled actin is stable for several months at $\leq -70^{\circ}\text{C}$, but only for a few days when thawed and stored at 4°C . AVOID FREEZING AND THAWING. Upon receipt, fluorescently labeled actin conjugates should be stored at $\leq -70^{\circ}\text{C}$, protected from light. Undiluted conjugates are stable for approximately 6 months when stored under these conditions. AVOID REPEATED FREEZING AND THAWING.

Properties

Molecular Probes' unlabeled actin is comprised of G-actin monomer, estimated to be >99% pure as determined by SDS polyacrylamide gel electrophoresis. The fluorescently labeled actin conjugates are labeled with approximately 1–2 fluorophore molecules per G-actin monomer. The degree of labeling for each lot is indicated on the product label.

Applications

Various methods for introducing actin or fluorescent actin conjugates into cells have been described in the literature. The most common technique involves microinjection,¹⁻⁵ although electroporation methods have also been described.⁶

We recommend centrifuging the unlabeled actin and fluorescently labeled actin conjugate solutions in a microcentrifuge before use; only the supernatant should then be added to the experiment. This step will eliminate any protein aggregates that may have formed during storage.

References

1. Cell Struct Funct 22, 59 (1997); 2. Development 103, 675 (1988); 3. J Cell Biol 102, 1074 (1986); 4. J Cell Biol 101, 597 (1985); 5. Proc Natl Acad Sci USA 75, 857 (1978); 6. Cell Struct Funct 21, 189 (1996).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
A-12375	actin from rabbit muscle	1 mg
A-12373	actin from rabbit muscle, Alexa Fluor® 488 conjugate *in solution*	200 µg
A-12374	actin from rabbit muscle, Alexa Fluor® 568 conjugate *in solution*	200 µg
A-34050	actin from rabbit muscle, Alexa Fluor® 594 conjugate *in solution*	200 µg
A-34051	actin from rabbit muscle, Alexa Fluor® 647 conjugate *in solution*	200 µg

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — www.probes.com — for the most up-to-date information.

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