Actin and Actin Conjugates

Catalog Numbers A12375, A12373, A12374, A34050

Pub. No. MAN0002095 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Unlabeled and fluorescently labeled actin have become important tools for investigating cytoskeleton dynamics in vivo (Shimada, 1997; Kellogg, 1988; Amato, 1986; Wang, 1985; Taylor, 1978). Invitrogen™ offers unlabeled actin from rabbit muscle, as well as fluorescently labeled actin conjugates prepared with our proprietary Alexa Fluor™ dyes (see table 1). These 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 (Kellogg, 1988). 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.

Various methods for introducing actin or fluorescent actin conjugates into cells have been described in the literature (Shimada, 1997; Kellogg, 1988; Amato, 1986; Wang, 1985; Taylor, 1978). The most common technique involves microinjection, although electroporation methods have also been described (Yumura, 1996).

Contents and storage

Contents	Amount[1]	Storage[2]
One of the following:		
Unlabeled actin	1 mg (Lyophilized from 1 mL of a solution of 2 mM Tris pH 8.0, and 0.2 mM ATP)	• ⩽ –20°C • Dessicated • Avoid repeated freeze-thaw cycles
Fluorescently labeled actin conjugate solution	200 µg (5–20 mg/mL in G buffer[3] containing 10% (w/v) sucrose)	• ⩽ –70°C • Protected from light • Avoid repeated freeze-thaw cycles

[1] The exact concentration of the protein is indicated on the product label.
[2] When stored as directed, the product is stable for ~6 months.
[3] 5 mM Tris pH 8.1, 0.2 mM $CaCl_2$, 0.2 mM dithiothreitol, and 0.2 mM ATP.

Table 1 Approximate absorbance and emission maxima for the actin conjugates

Actin[1] and conjugates	Cat. No.	Absorbance (nm)	Emission (nm)
Actin (unlabeled)	A12375	N/A	N/A
Alexa Fluor™ 488	A12373	495	519
Alexa Fluor™ 568	A12374	578	603
Alexa Fluor™ 594	A34050	590	617

[1] From rabbit muscle

Properties

Component	Composition	Purity
Unlabeled actin	G-actin monomer	>99% pure as determined by SDS-PAGE analysis
Fluorescently labeled actin	1–2 fluorophore molecules per G-actin monomer	The degree of labeling for each lot is indicated on the product label.

Prepare a 2 mg/mL stock solution of unlabeled actin

1. Add 0.5 mL of 2 mM Tris pH 8.0 directly to the vial of unlabeled actin, then mix.
2. Aliquot in small volumes, then store at ≤ –70°C.

   Note: Reconstituted unlabeled actin is stable for several months at ≤ –70°C, but only for a few days when thawed and stored at 4°C.

IMPORTANT! Avoid repeated freeze thawing.

Before each use

1. Centrifuge the unlabeled actin and fluorescently labeled actin conjugate solution in a microcentrifuge.
2. Use only the supernatant for your experiments.

   This prevents the use of protein aggregates that can form during storage.

IMPORTANT! Avoid repeated freeze thawing.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

References

Shimada Y, Suzuki H, Konno A. 1997. Dynamics of actin in cardiac myofibrils and fibroblast stress fibers. Cell Struct Funct. Feb;22(1): 59-64.

Kellogg DR, Mitchison TJ, Alberts BM. 1988. Behaviour of microtubules and actin filaments in living Drosophila embryos. Development. Aug;103(4):675-86.

Amato PA, Taylor DL. 1986. Probing the mechanism of incorporation of fluorescently labeled actin into stress fibers. J Cell Biol. Mar;102(3):1074-84.

Wang YL. 1985. Exchange of actin subunits at the leading edge of living fibroblasts: possible role of treadmilling. J Cell Biol. Aug; 101(2):597-602.

Taylor DL, Wang YL. 1978. Molecular cytochemistry: incorporation of fluorescently labeled actin into living cells. Proc Natl Acad Sci USA. Feb;75(2):857-61.

Yumura S. 1996. Spatial distribution of fluorescently labeled actin in living Dictyostelium amoebae. Cell Struct Funct. Jun;21(3): 189-97.

Manufacturer: Life Technologies Corporation | 29851 Willow Creek | Eugene, OR 97402

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0002095

Revision	Date	Description
A.0	24 August 2018	Update to the concentration of fluorescently labeled actin conjugates, and rebranding to a new document.

Important Licensing Information: This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

©2018 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.