

# ACTIN-TOOLKITS

Actin-Based Bioassays for  
Functional & Structural Ligand Analysis



Analytical Biochemistry

Molecular Cell Biology

Proteomics

Structural Biology

Molecular Medicine

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Notice to Purchaser:

For Use in Research Only.

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Handbook Actin-Toolkit Fluorescence Microscopy (Rhodamine-Actin Cat. #: 8080-01)

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## Kit Content

### Actin-Toolkit Fluorescence Microscopy

Rhodamine-Actin	4x100µg
Actin (0.5mg)	1x0.5ml
PolyMix <sup>1</sup>	1x1.0ml (1ml, 10x stock)
MonoMix <sup>2</sup>	5x50ml (1ml, 50x stock)
MgCl <sub>2</sub> (1M)	1x0.5ml
Tubes (light protective)	10x
Handbook	1

<sup>1</sup> Content: 1M KCl, 20mM MgCl<sub>2</sub>, 0.1M imidazole pH 7.4, 10mM ATP

<sup>2</sup> Content: 5mM Tris pH 8.2, 0.1mM CaCl<sub>2</sub>, 0.4mM ATP, 0.5mM DTT

## Shipping, Storage and Handling Conditions

Actin-Toolkits are shipped at ambient temperature. Proteins and ATP-containing buffers are stable for at least 3 months when frozen at –20°C and for at least 6 months when frozen at –70°C upon arrival. Avoid repeated freeze/thaw of ATP-containing components and of proteins. Solubilized proteins and ATP-containing solutions must be kept on ice and used as described in the Protocol Section.

## Product Warranty

Hypermol guarantees the quality and product performance described in this handbook only when products are frozen upon arrival as mentioned above. We do not take any guarantee for uses of our products other than described here. This product is designed for *in vitro* use only and not for use in diagnostic processes.

Should any product fail to perform as guaranteed due to reasons other than misuse or should not meet your expectations, please first contact our TechnicalService (techserv@hypermol.com or +49 521 987 623 0) within 5 working days and then return the product to Hypermol as advised. We reserve the right to test the performance of returned products in order to suggest replacement free of charge or refund of the purchase price. The buyer obtains a copy of our “Terms and Conditions of Sale” before ordering, and agrees to this by ordering.

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## Preface

Identification of an actin-binding protein is a major step forward in research. Today about two hundred proteins are known to either possess direct or cryptic binding sites for actin.

- Actin-Toolkits were developed to safely guide experiments with actin of highest quality.
- The handbook provides background information and protocols for successful and error-free handling.
- Actin-Toolkit proteins are fully biologically active.
- Actin-Toolkits are user-friendly all-in-one applications.

Actin Toolkits are invaluable tools to analyze the biological activity of protein ligands, especially for recombinant proteins, fragments or mutants. Identification and mapping of actin-binding sites in full length proteins or fragments are examples for the use of these assays.

The Actin-Toolkit Fluorescence Microscopy was developed to analyse and demonstrate the effect of ligands on actin polymerization, or ligand-induced formation of actin bundles and networks. In the assays Rhodamine-Actin – lysine-labelled actin by esterification - is mixed with the ligand to observe the effects on either G- or F-actin. In contrast to the staining with fluorochromated phalloidin, which can interfere with ligand binding sites on the actin molecule, no such observations were made with Rhodamine labelled actin.

## Introduction to Actin and the Actin Cytoskeleton

Actin is one of the most abundant proteins of eukaryotic cells. Comprising 5 to 10% of the total cellular protein, actin turned out to be a key protein of cellular architecture and thus keeper of cellular functions.

The most characteristic feature of single actin molecules (globular or G-actin,  $M_r=42\text{kD}$ , single polypeptide chain) is to polymerize into double helical filaments (filamentous or F-actin) of several micrometres length *in vitro* and *in situ*. This polymerization process is readily initiated at physiological salt concentrations in the presence of ATP.

Today more than two hundred proteins are known to possess one or more actin binding sites. Some proteins can readily bind to actin; some have to undergo ligand induced conformational changes to bind to actin. Several dozens of proteins directly modulate either the state or the conformation of F- or G-actin.

In addition to the filamentous actin incorporated into the cytoskeleton, cells have a rather variable pool of unpolymerized actin (30-50% of the total actin). Actin sequestering proteins like thymosin  $\beta_4$  take control of the G-actin pool, which would otherwise polymerize considering intracellular conditions.

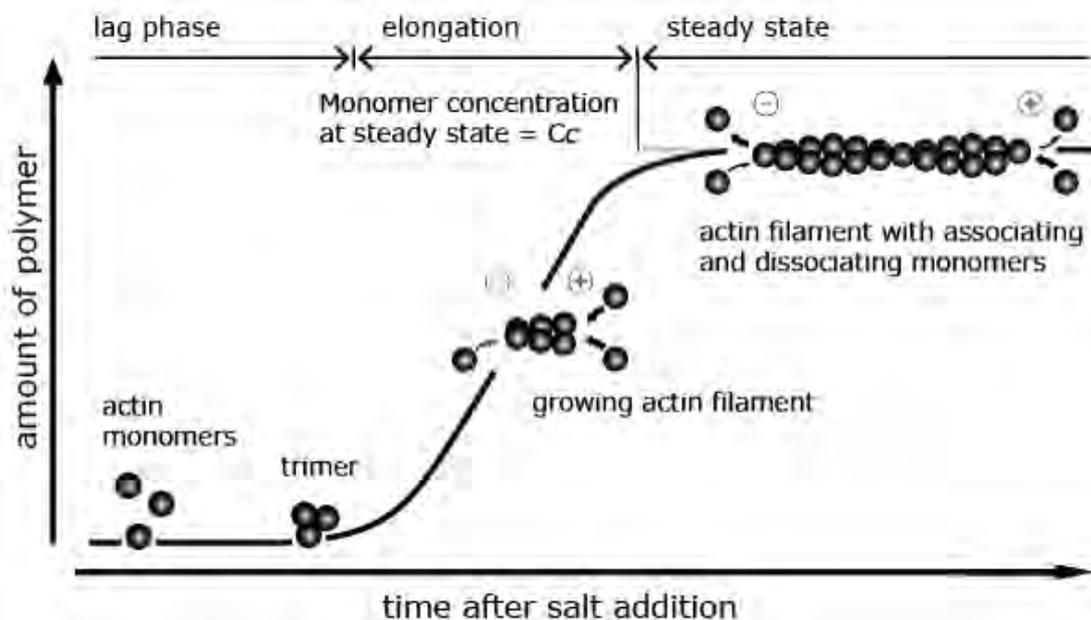
Apart from these direct actions on actin - like polymerization, nucleation, capping, depolymerization, severing, bundling etc. - the function of many actin-binding proteins is to support the different states of the actin-cytoskeleton in order to follow the demands of the cellular life (movement, cell division, signalling, etc.).

Due to its central function for the cell, it is not surprising, that the amino acid sequence of actin is highly conserved throughout evolution. Human skeletal muscle actin is practically identical in sequence to muscle actin in mouse, rat, rabbit, chicken, and beef.

## *In Vitro* Polymerization of Actin

An increase of the salt concentration of a low salt buffer (e.g. <math><5\text{mM}</math> KCl) in the presence of <math>0.1\text{mM}</math> ATP to a nearly intracellular salt concentration (<math>\sim 100\text{mM}</math> KCl, <math>2\text{mM}</math>  $\text{MgCl}_2$ ) induces the polymerization of G-actin to F-actin. In biochemical terms, the polymerization of actin is a non-covalent association of actin monomers. Each actin monomer (G-actin) has two actin binding sites and is incorporated into the double helical filament via head-to-tail association. The result of this polymerization is a polar, right handed double-stranded actin helix (synonym. actin filament) with a pitch of <math>73.6\text{nm}</math> per turn and a diameter of <math>\sim 8\text{nm}</math>.

The polymerization of actin can be divided into three steps. During the first step (nucleation), actin monomers form an unstable dimer, which becomes stabilized by the addition of another G-actin molecule to form a trimer. Actin-trimers strongly favour the elongation of the actin filament by further monomer addition. Both, temperature and concentration alter polymerization kinetics.



G-actin is an ATPase converting ATP in  $\text{ADP} \cdot \text{P}_i$ . Either ATP or ADP is bound in the nucleotide binding pocket of the actin molecule. As the affinity of actin is higher for ATP than for ADP, the ADP is exchanged. Free actin monomers hydrolyse ATP

at a very low rate. Upon polymerization the process of hydrolysis is significantly accelerated.

In the early stage of elongation the polarity of actin filaments becomes obvious. Actin polymerizes from both ends of the filament, but the rate of polymerization at either end is different. A fast growing end (plus-end or barbed end) and a slowly growing minus-end (pointed end) are distinguished. The polarity of actin

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## Protocol Section

### Protocol 1: Preparation of Rhodamine G-Actin

For the preparation of Rhodamine G-actin or F-actin, the Rhodamine G-actin powder has to be reconstituted to make a stock solution.

PROTECT Rhodamine-ACTIN STOCK SOLUTIONS FROM LIGHT!

1. To obtain a 1.1mg/ml (26 $\mu$ M) Rhodamine G-actin stock solution, add 90 $\mu$ l H<sub>2</sub>O to the tube containing actin, and vortex vigorously for 10secs.
2. Leave the protein solution to rehydrate for ~5min at room temperature, then vortex again (5secs) to dissolve the protein completely. Repeat the procedure until actin is dissolved.

## Protocol Section

### Protocol 2: Preparation of Rhodamine F-Actin

1. Prepare the Rhodamine G-actin stock solution according to Protocol 1.
2. Add 1.0ml of H<sub>2</sub>O to the tube containing PolyMix, and vortex for ~30secs to fully dissolve the PolyMix (10x PolyMix stock solution). Aliquot PolyMix 10x in portions of 250µl, store at -70°C and do not refreeze after thawing.
3. Add 10.0µl of PolyMix (10x) to the Rhodamine G-actin stock solution, which will then be adjusted to 1mg/ml (24µM) and mix immediately using a pipette.
4. Leave the polymerizing actin solution for 30min at room temperature

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## Protocol Section

### Protocol 3: Preparation of Rhodamine F-Actin for Bundles

In the following bundling assay, a positive control is prepared by adding a final concentration of 50mM MgCl<sub>2</sub> to the Rhodamine F-actin sample. Upon addition of high MgCl<sub>2</sub> concentrations F-actin forms bundles, the so called paracrystals, which are efficiently and easily observed by fluorescence microscopy. In combination with biochemical data from cosedimentation analysis (Actin-Toolkit F-Actin Binding Cat.#: 8010-01 / Cat.#: 8100-01), ligand induced bundling can be demonstrated.

ALWAYS PRE-PIPETTE THE VISCOUS RHODAMINE F-ACTIN SOLUTIONS TO

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## Protocol Section

<i>Control sample</i>	<i>Actin</i>	<i>1x PolyMix</i>	<i>MgCl<sub>2</sub></i>	<i>Final volume</i>
4. Rho F-actin + MgCl <sub>2</sub>	6µl	13µl	1µl	20µl

4. Leave the samples for 30-60min at room temperature.
5. Prepare the first sample slide and inspect under the microscopy, and then prepare the next sample slide. To prepare the slides use 8µl of the

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## Protocol Section

### Protocol 4: Ligand-Induced Polymerization and Bundling

Proteins that bundle F-actin are often capable to polymerize G-actin into F-actin. Using a laser-type microscope, actin filaments and can easily be resolved, while also less equipped epifluorescence microscopes resolve bundle formation. In the following approach bundles should be observed under non-polymerizing conditions, showing that G-actin was induced to polymerize to F-actin, and has been subsequently bundled.

## Protocol Section

5. Use 8 $\mu$ l of sample # 2., drop onto the slide, immediately place a clean coverslip on top, and use for microscopy. Do not allow the samples to dry.

Note: for time dependent observations addition of the ligand should be performed at the microscope. Start the timer upon addition of each component.

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## Troubleshooting Guide

Protein-  
concentration of  
ligand is low

Low ligand concentrations often require the addition of large volumes to the F-actin stock solution. An easy way to compensate low ligand concentrations is to prepare a Rhodamine G-actin stock of 2 or 3mg/ml, by adding 34 $\mu$ l or 22.5 $\mu$ l of H<sub>2</sub>O to G-actin according to Protocol 1, then polymerize. After incubation of actin and ligand the sample should be diluted for fluorescence microscopy to 0,2-

are tolerated by F-actin. Adjust the salt concentration in the reference samples.

Bundling is not observed

Perform the control experiments described in Protocol 3. In the presence of 50mM  $MgCl_2$  bundles are formed and seen under the fluorescence microscope. Inspect the samples slowly to match the layer with bundles. For help, please [contact@HYPERMOL.com](mailto:contact@HYPERMOL.com)

## Toolkit Ordering Information

Product	Description	Cat. #	Size
Actin-Toolkit F-Actin Binding ( $\alpha$ -skeletal muscle actin or $\alpha$ -cardiac actin)	Determination and quantification of F-actin binding or bundling by ligands in solution.	8010-01	4x1.0mg Rabbit skeletal muscle actin
Actin-Toolkit G-Actin Binding ( $\alpha$ -skeletal muscle actin or $\alpha$ -cardiac actin)	Determination and quantification of ligands binding to ActinBeads in solution.	8020-01	4x250 $\mu$ l Rabbit skeletal muscle actin
Actin-Toolkit Fluorometry ( $\alpha$ -skeletal muscle actin)	Kinetic measurements of actin dynamics in solution based on pyrenyl fluorescence of actin.	8030-01	8x1.0mg Rabbit skeletal muscle actin
Actin-Toolkit TIRFM ( $\alpha$ -skeletal muscle actin or $\alpha$ -cardiac actin)	Single molecule imaging of ligands interacting with ATTO-fluorescent G- or F-actin.	8093-01	4x100 $\mu$ g Rabbit skeletal muscle actin
Actin-Toolkit Crystallography ( $\alpha$ -skeletal muscle actin)	Co-crystallization of a ligand with non-polymerizable, native G-actin.	8050-01	8x1.0mg Rabbit skeletal muscle actin
Actin-Toolkit SPR ( $\alpha$ -skeletal muscle actin or $\alpha$ -cardiac actin)	A unique method to analyze ligand interactions with actin filaments by surface plasmon resonance.	8090-01	4x250 $\mu$ g Rabbit skeletal muscle actin
Actin-Toolkit ELISA ( $\alpha$ -skeletal muscle actin or $\alpha$ -cardiac actin)	Molecular imaging analysis of ligands bound to monomeric actin, filaments or networks by TEM.	8070-01	4x0.5mg Rabbit skeletal muscle actin
Actin-Toolkit Fluorescence Microscopy ( $\alpha$ -skeletal muscle actin or $\alpha$ -cardiac actin)	Identification of ligands bundling actin filaments or forming filament networks by using ATTO-fluorescent actin.	8080-01	4x100 $\mu$ g Rabbit skeletal muscle actin

For further Toolkits and more product information we welcome your visit on

[www.hypermol.com](http://www.hypermol.com)

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We welcome your requests and questions!

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