ACTIN-TOOLKITS

Actin-Based Bioassays for
Functional & Structural Ligand Analysis

Analytical Biochemistry

Molecular Cell Biology

Proteomics

Structural Biology

Molecular Medicine

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Contents

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Handbook Actin-Toolkit: Electronmicroscopy

All products from HYPERMOL are made in Germany.

Kit Content

Actin-Toolkit Electron Microscopy Cat.# 8070-01 / Cat.# 8700-01

Actin	4x0.5mg
PolyMix (10x) [1]	2x1ml
MonoMix [2]	3x50ml
$MgCl_2$ (1M)	1x500µl
SpreadingSolution [3]	1x500µl
Filter Units (0.2µM)	4x
Handbook	1x

[1] Content: 1M KCl, 20mM $MgCl_2$, 0.1M imidazole pH 7.4, 10mM ATP, [2] Content: 5mM Tris pH 8.2, 0.2mM $CaCl_2$, 1mM ATP, 0.1mM DTT, [3] Content : 0.5mg/ml Bacitracin

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 ++495219876230) 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.

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 Electron Microscopy is ready-to use for the preparation electron microscopic specimen, hence making this method accessible for researchers who would like to get introduced to EM works on actin imaging. This Toolkit is designed for users that either took a short introduction at an EM facility or would pass kit and ligands for inspection to an EM service.

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, Mr=42kD, single polypeptide chain) is to polymerize into double helical filaments (filamentous or F-actin) of several micrometers 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 directly bind to actin; some have to undergo ligand induced conformational changes to bind to actin. Several dozens of proteins 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 β4 take control of the G-actin pool, which would otherwise polymerize considering intracellular conditions.

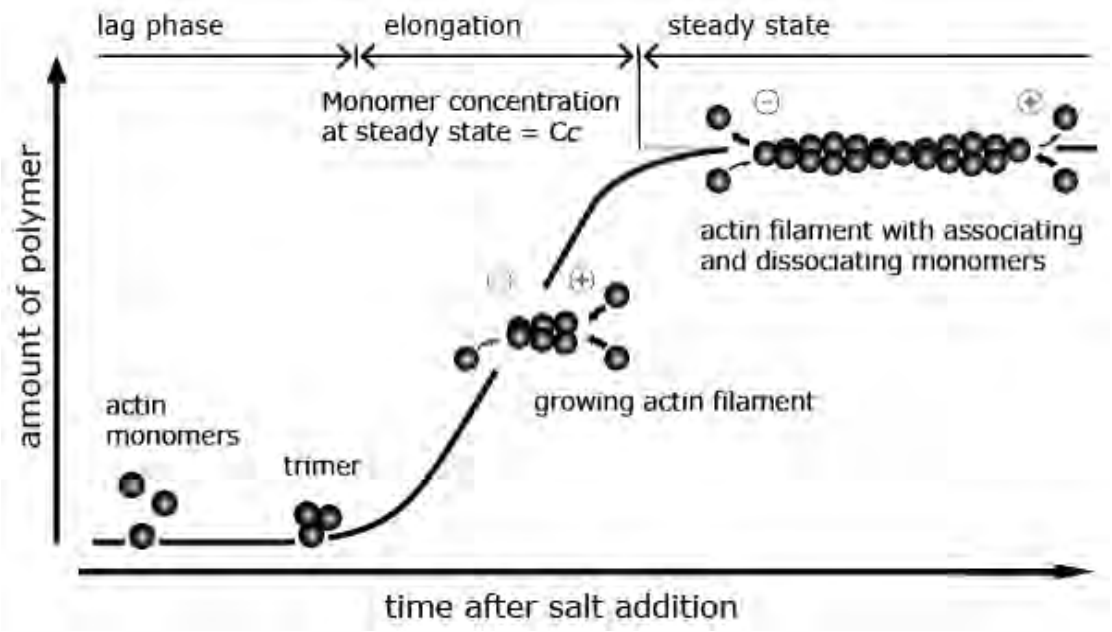
Apart from these direct actions on actin - like polymerization, nucleation, capping, depolymerization, severing, bundling etc. - the function of many actin-binding proteins is to support the different states of the actin-cytoskeleton in order to follow the demands of the cellular life (movement, cell 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. <5mM KCl) in the presence of 0.1mM ATP to a nearly intracellular salt concentration (~100mM KCl, 2mM $MgCl_2$) 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 several 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 73.6nm per turn and a diameter of ~8nm.

The polymerization of actin is 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 ADP*Pi. 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 hydrolyze 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 filaments is the consequence of the head-to-tail association of the monomers. The critical concentration (C_c) for polymerization is ~0.1µM for the plus-end and ~0.6µM for the minus-end. As a consequence, addition of monomers to the plus-end occurs below the C_c of the minus-end and thus filaments are still growing. Below the C_c actin does not polymerize. At the C_c actin monomers and filaments are in steady state, and F-actin is preferentially ADP-actin.

A typical phenomenon of the steady state is treadmilling, where monomers add to the plus-end while others dissociate from the minus-end. For actin alone, the equilibrium is a dynamic exchange of monomers between the G-actin and the F-actin pool. This dynamic is modulated by ABPs (actin-binding proteins).

At physiological salt concentration in the presence of ATP, two factors should be noted influencing the actin polymerization in the absence of ABPs: the actin-concentration and the state of the bound nucleotide.

Means for the choice of experimental conditions

✓ **buffer control**

Add salt and ATP. Polymerization buffers should contain about 0.1M KCl, an excess of 1mM ATP, and 2mM $MgCl_2$.

✓ **time control**

Wait 15-30min until polymerization reaches steady state.

✓ **temperature control**

Work at room temperature. Polymerization kinetics is usually monitored at 25°C.

Protocol Section

Protocol 1: Reconstitution of G-Actin

To make either G- or F-actin, the G-actin powder has to be reconstituted first.

1. To obtain a 1.0mg/ml (24µM) actin stock solution, add 500µl H_2O to the tube with actin, and vortex vigorously for 10secs.
2. Leave the protein solution to rehydrate for 1-2min at room temperature,

Storage of MonoMix

Keep reconstituted MonoMix on ice and use within 3-5 days. Freshly reconstituted MonoMix can be aliquoted (1.0ml) for flash freezing and stored at -70°C.

Protocol Section

Protocol 2: Preparation of F-Actin

Prepare an F-actin stock solution of 0.5mg/ml (12µM) at a final volume of 0.5ml.

1. Prepare a G-actin stock solution according to Protocol 1.

Storage of F-Actin

Always keep F-actin stock solution on ice and use at best within 2 weeks. The stability of F-actin stock solutions prepared according to protocol 1 is usually about 3 weeks.

DO NOT FREEZE F-ACTIN SOLUTIONS!

Protocol Section

Protocol 3: Negative staining of actin filaments and bundles

This method is used to characterize the effect of ligands binding to F-actin. The following 0.2µM filtered solutions should be prepared: 0.2ml of 1% Uranyl Acetate (RT) and 5ml of 1xPolyMix (on ice). Excellent results are obtained when specimen are prepared on glow discharged Formvar coated 300mesh Ni-grids with a thin carbon layer.

5. Remove the drop carefully from the edge of the grid using filter paper.
6. Wash with one drop of 1xPolyMix for 10secs, remove PolyMix and repeat washing twice. Exchange PolyMix quickly to avoid the specimen to turn dry.
7. After removal of PolyMix add 30µl of 1% (Uranyl Acetate for negative

Protocol Section

Negative staining of G-actin

Negative staining of G-actin is essentially performed as described in protocol 3. Instead of using PolyMix for washing the only and important difference is that MonoMix must be used to wash the specimen as only MonoMix maintains the monomer state of actin.

Troubleshooting Guide

Ligand-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 G-actin stock of 2 or 3mg/ml, by adding 0.45ml or 0.3ml of H_2O to 1mg of G-actin according to Protocol 1.
Ligand Buffer is not compatible with actin buffers.	Phosphate buffers are not compatible with G-actin and should not be used for F-actin either. The best way to

High protein concentration of the specimen.	Samples for electron microscopy should have a final actin concentration of 0.3mg/ml. The sample mix can be diluted to 0.1mg/ml with 1xPolyMix if required, but not less.
Actin filaments are too short or too long.	In the absence of ABPs the filament lengths depends on 1. the actin concentration and 2. the salt conditions. Actin solutions contain filaments of heterogeneous lengths. Due to the F-actin dynamic, it is not possible to adjust the filament population to a defined length in the absence of ABPs. As a rule of thumb, actin solutions of ~1mg/ml contain more short filaments (~0.5-5µm) and those of

Toolkit Ordering Information

Product	Description	Cat. #	Size
Actin-Toolkit F-Actin Binding (α-skeletal muscle actin or α-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 (α-skeletal muscle actin or α-cardiac actin)	Determination and quantification of ligands binding to ActinBeads in solution.	8020-01	4x250µl Rabbit skeletal muscle actin
Actin-Toolkit Fluorometry (α-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 (α-skeletal muscle actin or α-cardiac actin)	Single molecule imaging of ligands interacting with ATTO-fluorescent G- or F-actin.	8093-01	4x100µg Rabbit skeletal muscle actin
Actin-Toolkit Crystallography (α-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 (α-skeletal muscle actin or α-cardiac actin)	A unique method to analyze ligand interactions with actin filaments by surface plasmon resonance.	8090-01	4x250µg Rabbit skeletal muscle actin
Actin-Toolkit ELISA (α-skeletal muscle actin or α-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 (α-skeletal muscle actin or α-cardiac actin)	Identification of ligands bundling actin filaments or forming filament networks by using ATTO-fluorescent actin.	8080-01	4x100µg Rabbit skeletal muscle actin

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HYPERMOL
Schulenburger Landstr. 132A
D-30165 Hannover
Germany

mail: hypermol@hypermol.com
fon : ++49 52 19 87 62 26
fax : ++49 52 19 87 62 27

Mail Address: Hypermol | P.O. Box | 201025 D-33549 Bielefeld | Germany