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

Kit Contents 2
Shipping, Storage and Handling Conditions 2
Product Warranty 2
Preface 3

Introduction to Actin and the Actin Cytoskeleton 4
In Vitro Polymerization of Actin 5
Experimental Conditions 6

Protocol Section
Protocol 1: Buffer Setup 7
Protocol 2: Preparation of G-Actin 7
Storage of G-Actin 8
Protocol 3: Preparation of short actin filaments (SAF) 8
Storage of Ankerprotein™ and SAF 9
Protocol 4: Analyte-Actin Interaction 9
SPR Sample Preparation Scheme 12
Protocol 5: Removal of SAF from SA sensor chips 13
Ordering Information 14
Hypermol Services 15
Notes 16

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For Use in Research Only (RUO).
For *In Vitro* Use Only.
Not for Use in Human or Animal Diagnostic or Therapeutic Processes.

Handbook Actin-Toolkit: SPR

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Kit Content

Actin-Toolkit SPR
Cat.# 8090-01 / Cat.# 8900-01

Component	Amount
Actin	4x250 µg
Ankerprotein™	4x10 µg
FAB-P[1]	2x250 ml
GAB-P[2]	1x250 ml
PolyMix[3]	1x1ml (10x stock sol.) each
Supplements (ATP & DTT)	1x unit each
ReconBuffer	1x1ml
Phalloidin 100µM	1x250µl
Handbook	1x

[1] 100mM KCl, 2mM $MgCl_2$, 0.4mM ATP, 10mM imidazole pH 7.4, 0.05% Surfactant P20
[2] 0.1mM $CaCl_2$, 0.2mM DTT, 0.4mM ATP, 2mM Tris pH 8.2

Shipping, Storage and Handling Conditions

Actin-Toolkits are shipped at ambient temperature. All ready-to-use liquids without ATP and/or DTT can be stored at +4°C. After supplementation with ATP and/or DTT they are stored on ice for 1 week until use. Proteins, ATP and DTT are stored at −70°C upon arrival. 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 or therapeutic processes.

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.

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 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 β4 take control of the G-actin pool, which would otherwise polymerize considering intracellular conditions.

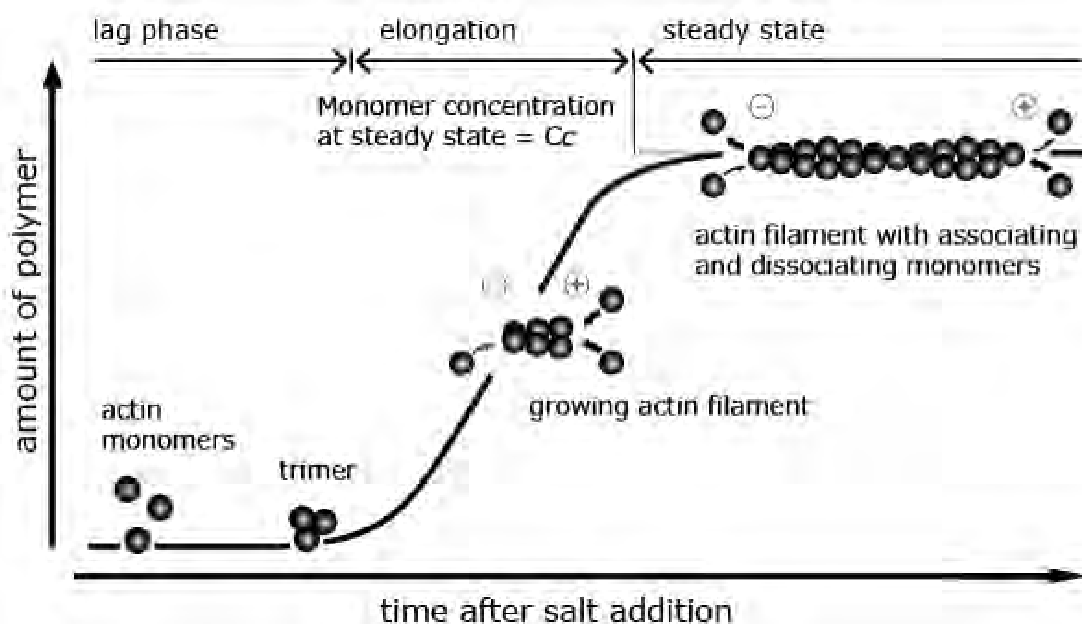
Apart from these direct actions on actin - like polymerization, nucleation, capping, depolymerization, severing, bundling etc. - the function of many actin-binding proteins is to support the different states of the actin-cytoskeleton in order to follow the demands of the cellular life (movement, cell 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 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 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 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 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 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, 0.4mM ATP, and 2mM $MgCl_2$. ATP-buffers must be kept on ice should be used within 5 days.

✓ **time control**

Wait 15-30min until polymerization reaches steady state.

✓ **temperature control**

Work at room temperature. Polymerization kinetics and binding studies are usually monitored at 25°C.

Protocol Section

Protocol 1: Buffer Setup

The kit contains 3 vials with ATP each for 250ml buffer. Start dissolving 2 of 3 vials and store the remaining vial at -70°C until use.

- ATP: add 1ml H_2O to each vial and vortex until dissolved (100mM stock).

Protocol Section

2. Leave the protein solution to rehydrate for 5min at room temperature, then vortex again (5secs) to dissolve the protein completely.

3. If possible, spin the G-actin solution 100.000xg, 1h and use the supernatant for experiments, or spin at low speed 15.000xg for 1h in a table-top centrifuge and use the supernatant. Transfer the supernatant

Protocol Section

4. Immediately transfer the above mixture to the tube with 250µl G-actin and vortex 2-3 seconds for mixing. You are now preparing a 0.5mg/ml (11.9µM) actin solution which is polymerizing into short actin filaments, which shall serve as a stock solution for dilution in the following step.

Protocol Section

2. For capturing prepare 0.5ml of a 0.5mg/ml SAF stock solution as described in protocol 3. Dilute the F-actin stock for capturing as follows:

   A. Mix 37.5µl PolyMix (10x) with 337.5µl H_2O to make 375µl of 1x PolyMix.

   B. Add 375µl to 250µl of the 0.5mg/ml F-actin stock solution to

Protocol Section

4. After the SAF have been immobilized and the surface has been sufficiently washed to give a stable baseline, the ligand (analyte) is introduced. Again, it is recommended to use a flow rate of 3µl/min for all flow cells. Compare binding of fc2 (SAF) and fc3 (with SAF-phalloidin).

   If both approaches (SAF and SAF-phalloidin) show binding of your

Sample Preparation Scheme

Protocol Section

Protocol 5: Removal of SAF from the SA sensor chip

The biotin-streptavidin interaction is very strong as characterized by its high dissociation constant (K_d= ~10^{-14} mol/L). Accordingly, the proteins are difficult – if not impossible - to remove from the SA chip. Wash with GAB-P (100µl/min, 20min) to depolymerize the actin filaments. It is useful to add 1mM EDTA to GAB-

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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My notes