

Version 3a, Last updated 9 June 2023

ab239724 Actin Polymerization/Depolymerization Assay Kit

For the detection of Actin in various biological samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

Actin Polymerization/Depolymerization Assay Kit (ab239724) can be used to study the effect of different compounds, proteins and tissue extracts on Actin polymerization and depolymerization.

The kit utilizes a proprietary Pyrene-labeled Actin molecule that develops a higher fluorescent signal if it undergoes polymerization. The signal can be easily detected using a fluorescence microplate reader. The assay is simple, high-throughput compatible, and can be completed in less than three hours.

Samples: Protein, Tissue Extracts, Compounds/Chemotherapeutic Agents.

2. Protocol Summary

Actin polymerization assay

Prepare all controls and samples according to the protocol.



Mix well and incubate for 15 minutes.



Add 10 μ L of Supplemented Buffer G and 10 μ L of Supplemented Buffer P to the background and positive control respectively.



Measure Fluorescence Ex/Em: 365/410 nm

Actin Depolymerization assay

Prepare all controls and samples according to the protocol



Incubate the plate at room temperature for one hour to polymerize Actin protected from light



For the negative control add 10 μ L of the solvent of test sample/ Supplemented Buffer G.



Measure Fluorescence Ex/Em: 365/410 nm

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
Buffer G	20 mL	-20°C
10X Buffer P/Buffer P (10X)	1.5 mL	-20°C
Labelled Rabbit Muscle Actin/Labeled Rabbit Muscle Actin	4 vials	-20°C
ATP Stock (100 mM)/ATP (100 mM)	2 x 100 µl	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well plate with flat bottom; clear or opaque bottoms are both acceptable. Black-walled plates are recommended for this assay
- Multi-well spectrophotometer

- DTT

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 Buffer G:

Store at -20°C. Supplement Buffer G with 0.2 mM ATP and 0.5 mM DTT (For example: add 2 µl of ATP Stock (100mM)/100 mM ATP, and 5 µl of 100 mM DTT to 993 µl of Buffer G). Prepare as needed. (DTT is not provided).

6.2 10X Buffer P/Buffer P (10X):

Store at -20°C. Supplement Buffer P with 10 mM ATP (for example: add 10 µl of ATP Stock (100mM)/100 mM ATP per 90 µl of 10X Buffer P). Avoid multiple freeze thaw cycles. Prepare as needed.

6.3 Labelled Rabbit Muscle Actin/Labeled Rabbit Muscle Actin:

Store at -20°C. Keep the tubes in dark to avoid photobleaching. Reconstitute vial as needed.

Before experiment, reconstitute the contents of one vial with 500 µl of supplemented Buffer G.

After reconstitution, keep the tube on ice for 1 hour. Once reconstituted, Actin can be flash frozen and saved at -80C up to 1 week.

Stored actin may lose activity by 30%. Use lyophilized Actin within three months. Avoid multiple freeze thaws.

6.4 ATP Stock (100mM)/ATP (100 mM):

Ready to use. Store at -20° C. Thaw and aliquot before use. Avoid multiple freeze thaw cycles.

7. Assay Procedure

7.1 Actin Polymerization Assay:

Δ Note: Avoid exposing labeled Actin to light for extended periods of time. Protect labeled actin from light.

Δ Note: Actin Polymerization/Depolymerization experiments use Buffer G supplemented with ATP and Buffer P supplemented with ATP (See Section 6; Reagent Preparation). For brevity, these buffers will be referred as Supp. Buffer G and Supp. Buffer P respectively.

7.1.1 Prepare sample background, positive and sample on a black 96-well microplate following the table below:

	Sample Background control	Positive Control	Sample
Supp. Buffer G	70 μ L	70 μ L	60 μ L
Actin	20 μ L	20 μ L	20 μ L
Test sample	-	-	10 μ L

7.1.2 Mix well. Incubate microplate for 15 mins, or preferred incubation time based on your protocols at room temperature.

7.1.3 After incubation, **For Background Control:** add 10 μ L of Supp. Buffer G; **For Positive Control** and **Sample Test:** add 10 μ L of Supp. 10X Buffer P/Buffer P (10X) to each well containing samples and positive control. Mix and then start data acquisition (see step 3).

Δ Note: If the initial signal is too high, incubate on ice in dark for 1 hour.

7.2 Actin Depolymerization Assay:

7.2.1 First, to make polymerized Actin (F Actin), incubate Actin, Supp. Buffer P, Supp. Buffer G, test sample(s) based on the following table:

	Negative control	Sample
Supp. 10X Buffer G	60 μ L	60 μ L
Supp. 10X Buffer P (10X)	10 μ L	10 μ L
Actin	20 μ L	20 μ L

7.2.2 Incubate the plate at room temperature for one hour to polymerize Actin protected from light. To make sure that the polymerization is complete, you can take a measurement after 1 hour.

7.2.3 For Negative Control: add 10 μ l of the solvent of test sample/Supp. Buffer G. For Sample: add 10 μ l of test sample, start data acquisition (see step 3).

7.3 Measurement

7.3.1 Measure Fluorescence Ex/Em: 365/410 nm in kinetic mode for 1 hr. at room temperature.

7.3.2 Choose two time points (t_{FINAL} & t_{INITIAL}) in the linear range of the plot and obtain the corresponding values for the fluorescence ($\text{RFU}_{\text{FINAL}}$ and $\text{RFU}_{\text{INITIAL}}$).

7.3.3 For Actin Polymerization assay calculate $(\text{RFU}_{\text{FINAL}} - \text{RFU}_{\text{INITIAL}})/\Delta t$ and for Actin Depolymerization assay calculate $(\text{RFU}_{\text{INITIAL}} - \text{RFU}_{\text{FINAL}})/\Delta t$.

8. Data Analysis

To calculate the effect of test sample on Actin polymerization and/or Actin depolymerization, calculate ΔRFU_P , ΔRFU_G and ΔRFU_S as indicated in the following equations:

ΔRFU_G = Generated fluorescence of Actin in presence of Buffer G (monomeric actin)

ΔRFU_P = Generated fluorescence of Actin in presence of Buffer P (polymeric actin)

ΔRFU_S = Generated fluorescence of Actin with test sample

$$\text{Percentage Activation Effect} = \left(\frac{\Delta RFU_S}{\Delta RFU_P} \right) \times 100$$

$$\text{Percentage Inhibition Effect} = \left(\frac{\Delta RFU_S}{\Delta RFU_G} \right) \times 100$$

9. Typical Data

Typical data provided for demonstration purposes only.

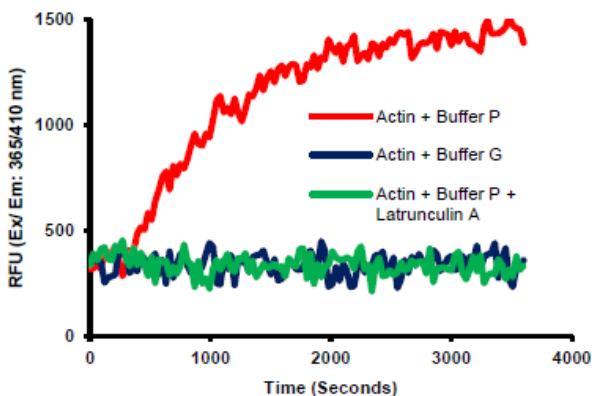


Figure 1. Actin Polymerization is induced by Buffer P. The process is inhibited by Latrunculin A (23 μ M). Assays were performed following the kit protocol. Note: Latrunculin A is Actin polymerization inhibitor in vitro and in vivo by the formation of a 1:1 complex with monomeric G-actin. Latrunculin A acts a depolymerization agent acting on Actin filaments (F-actin).

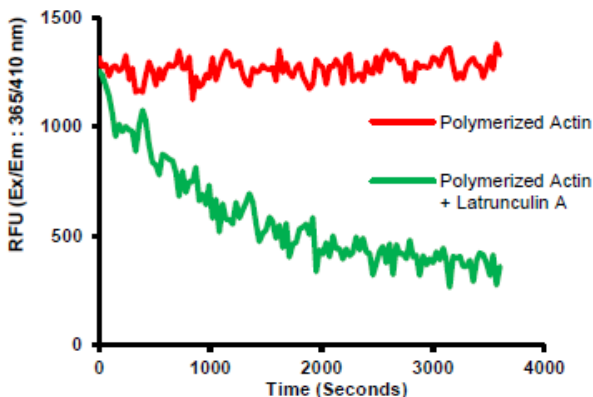


Figure 2. Polymerized Actin is depolymerized by Latrunculin A (23 μ M). Assays were performed following the kit protocol. Note: Latrunculin A is Actin polymerization inhibitor in vitro and in vivo by the formation of a 1:1 complex with monomeric G-actin. Latrunculin A acts a depolymerization agent acting on Actin filaments (F-actin).

10. Notes

Technical Support

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