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Actin Polymerization/Depolymerization Assay Kit (Fluorometric)

rev 11/20

(Catalog # K457-100; 100 assays; Store kit at -20 °C)

I. Introduction:

Actin, a highly conserved and abundant protein in eukaryotic cells, is one of the major components of cytoskeleton. It can be found as monomeric globular protein, called G actin or it can polymerize into filamentous actin, named F actin. Actin plays major roles in cell division, cell motility, cell signaling, organelle movement, etc. Mammals have 6 isoforms of Actin, which can be divided into 3 classes, α, β and γ. Muscle Actin is α class and all other non-muscle actins belong to β and γ -classes. Understanding the effect of different drugs, proteins, etc. on Actin Polymerization and Depolymerization is very important for understanding cellular machinery. More importantly because Cytoskeleton is a very important target for cancer therapy. **BioVision's Actin Polymerization/Depolymerization Assay Kit** 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.

Labeled Rabbit Muscle Actin (G actin) —**Buffer P/ Polymerizing Agent**→ Polymerized Actin (F Actin); higher fluorescence (Fluorescence: Ex/ Em = 365/410 nm)

Polymerized Actin (F Actin) —**Depolymerizing Agent**→ Depolymerized Actin with lower fluorescence (Fluorescence: Ex/ Em = 365/410 nm)

II. Applications:

- Study and quantitate the effect of different compounds, proteins and tissue extracts on Actin polymerization and/or depolymerization.
- Evaluation of critical concentrations of actin polymerization in different conditions.

III. Sample Type:

- Protein, Tissue Extracts, Compounds/Chemotherapeutic Agents.

IV. Kit Contents:

Components	K457-100	Cap Code	Part Number
Buffer G	20 ml	WM	K457-100-1
Buffer P (10X)	1.5 ml	Clear	K457-100-2
Labeled Rabbit Muscle Actin	4 vials	Blue	K457-100-3
ATP (100 mM)	2 X 100 µl	Yellow	K457-100-4

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. Black plates are required for this assay.
- Multi-well fluorescence microplate reader.
- DTT

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the assay**.**

- **Buffer G:** Store the buffer at -20 ºC. Supplement Buffer G with 0.2 mM ATP and 0.5 mM DTT (For example: add 2 µl of 100 mM ATP, and 5 µl of 100 mM DTT to 993 µl of Buffer G). Prepare as needed. (DTT is not provided).
- **Buffer P (10X):** Store the buffer at -20 ºC. Supplement Buffer P with 10 mM ATP (for example: add 10 µl of 100 mM ATP per 90 µl of 10X Buffer P). Avoid multiple freeze thaw cycles. Prepare as needed.
- **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 re-constituted, 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.
- **ATP (100 mM)**: Ready to use. Store at -20 ºC. Thaw and aliquot before use. Avoid multiple freeze thaw cycles.

VII. Actin Polymerization/Depolymerization Assay Protocol:

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

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

1. **Actin Polymerization Assay:** Prepare sample background, positive and sample on a black 96 microplate following the table below:

	Sample Background Control	Positive Control	Sample
Supp. Buffer G	70 µl	70 µl	60 µl
Actin	20 µl	20 µl	20 µl
Test Sample	-	-	10 µl

Mix well. Incubate microplate for 15 min, or preferred incubation time based on your protocols at room temperature (RT). After incubation, **For Background Control:** add 10 µl of Supp. Buffer G; **For Positive Control and Sample Test:** Add 10 µl of Supp. Buffer P (10X) to each well containing samples and positive control. Mix and then start data acquisition (see step 3).

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

2. **Actin Depolymerization Assay:** 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 µl	60 µl
Supp. Buffer P (10X)	10 µl	10 µl
Actin	20 µl	20 µl

Incubate the plate at RT for one hr to polymerize Actin protected from light. To make sure that the polymerization is complete, you can take a measurement after 1 hour. **For Negative Control**: add 10 µl of the solvent of test sample/Supp. Buffer G. **For Sample:** add 10 µl of test sample, start data acquisition (see step 3).

3. **Measurement:** Measure Fluorescence Ex/Em = 365/410 nm in kinetic mode for 1 hr. at RT. Choose two time points (t_{FINAL} & $t_{INITIAL}$) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_{FINAL} and $RFU_{INITIAL}$). For Actin Polymerization assay calculate (RFU_{FINAL} - $RFU_{INITIAL}$)/Δt and for Actin Depolymerization assay calculate ($RFU_{INITIAL}$ – RFU_{FINAL})/Δt.

4. **Calculation:** 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)

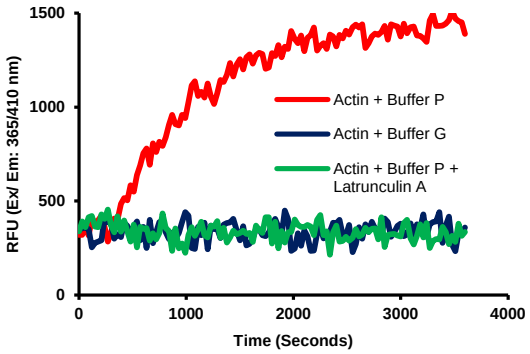
ΔRFU_p = Generated fluorescence of Actin in presence of Buffer P (polymeric actin)

ΔRFU_s = Generated fluorescence of Actin with test sample

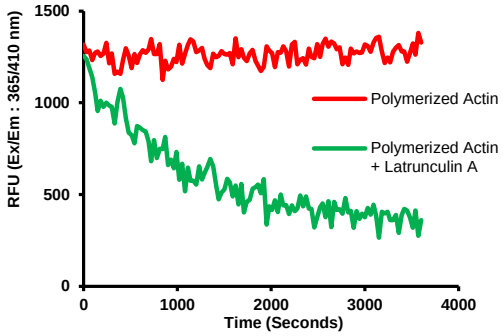
Percentage Activation Effect = $\left(\frac{\Delta RFU_S}{\Delta RFU_P}\right) \times 100$

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

a)



b)



Figures: (a) **Actin Polymerizarion:** Actin Polymerization is induced by Buffer P. The process is inhibited by Latrunculin A (23 µM) (b) **Actin Depolymerization:** Polymerized Actin is depolymerized by Latrunculin A (23 µM). 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 (Factin).

VIII. Related Products:

Latrunculin A (2181)
Latrunculin B (2182)
Actin, Rabbit Muscle

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