

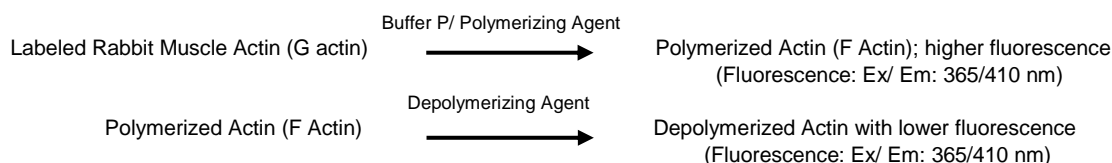
## Actin Polymerization/Depolymerization Assay Kit (Fluorometric)

Rev 3/19

(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,  $\alpha$ ,  $\beta$  and  $\gamma$ . Muscle Actin is  $\alpha$  class and all other non-muscle actins belong to  $\beta$  and  $\gamma$  -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.



### 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	Green	K457-100-3
ATP (100 mM)	2 X 100 $\mu$ l	Yellow	K457-100-4

### V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White 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 entire protocol before performing the assay.

- **Buffer G:** Store buffer at -20°C. Supplement Buffer G with 0.2 mM ATP and 0.5 mM DTT (For example: add 2  $\mu$ l of 100 mM ATP, and 5  $\mu$ l of 100 mM DTT to 993  $\mu$ l of Buffer G). Prepare as needed. (DTT is not provided).
- **Buffer P (10X):** Store buffer at -20°C. Supplement Buffer P with 10 mM ATP (for example: add 10  $\mu$ l of 100 mM ATP per 90  $\mu$ l 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  $\mu$ l 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° C. 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 white 96 microplate following the table below:

	Sample Background Control	Positive Control	Sample
Supp. Buffer G	70 $\mu$ l	70 $\mu$ l	60 $\mu$ l
Actin	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l
Test Sample	-	-	10 $\mu$ l

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Mix well. Incubate microplate for 15 mins, or preferred incubation time based on your protocols at room temperature. After incubation, **For Background Control:** add 10  $\mu$ l of Supp. Buffer G; **For Positive Control and Sample Test:** add 10  $\mu$ l 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 hour.

**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 $\mu$ l	60 $\mu$ l
Supp. Buffer P (10X)	10 $\mu$ l	10 $\mu$ l
Actin	20 $\mu$ l	20 $\mu$ l

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. **For Negative Control:** add 10  $\mu$ l of the solvent of test sample/Supp. Buffer G. **For Sample:** add 10  $\mu$ l 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 room temperature. Choose two time points ( $t_{\text{FINAL}}$  &  $t_{\text{INITIAL}}$ ) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU<sub>FINAL</sub> and RFU<sub>INITIAL</sub>). For Actin Polymerization assay calculate (RFU<sub>FINAL</sub> - RFU<sub>INITIAL</sub>)/ $\Delta t$  and for Actin Depolymerization assay calculate (RFU<sub>INITIAL</sub> - RFU<sub>FINAL</sub>)/ $\Delta t$ .

**4. Calculation:** To calculate the effect of test sample on Actin polymerization and/or Actin depolymerization, calculate  $\Delta$ RFU<sub>P</sub>,  $\Delta$ RFU<sub>G</sub> and  $\Delta$ RFU<sub>S</sub> as indicated in the following equations:

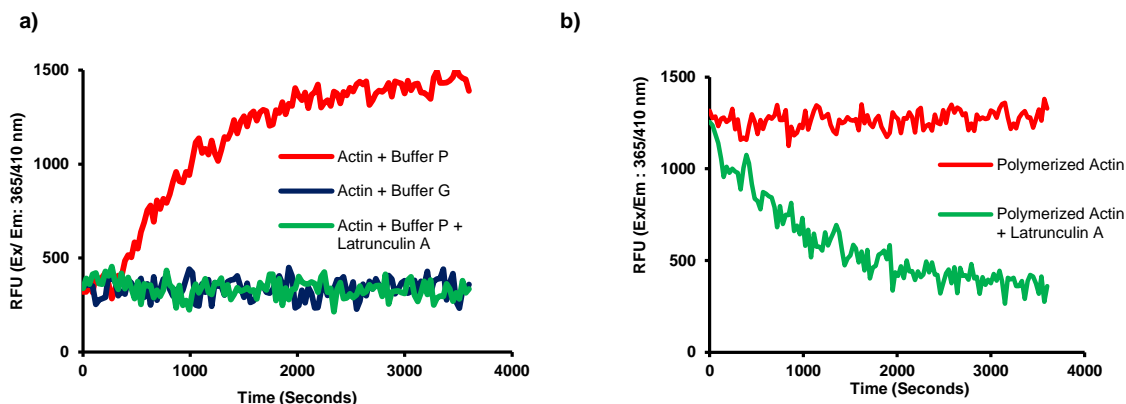
$\Delta$ RFU<sub>G</sub> = Generated fluorescence of Actin in presence of Buffer G (monomeric actin)

$\Delta$ RFU<sub>P</sub> = Generated fluorescence of Actin in presence of Buffer P (polymeric actin)

$\Delta$ RFU<sub>S</sub> = Generated fluorescence of Actin with test sample

$$\text{Percentage Activation Effect} = \left( \frac{\Delta \text{RFU}_S}{\Delta \text{RFU}_P} \right) \times 100$$

$$\text{Percentage Inhibition Effect} = \left( \frac{\Delta \text{RFU}_S}{\Delta \text{RFU}_G} \right) \times 100$$



**Figures:** (a) **Actin Polymerization:** Actin Polymerization is induced by Buffer P. The process is inhibited by Latrunculin A (23  $\mu$ M) (b) **Actin Depolymerization:** Polymerized Actin is depolymerized by Latrunculin A (23  $\mu$ 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).

#### VIII. RELATED PRODUCTS:

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

**FOR RESEARCH USE ONLY! Not to be used on humans.**