Actin Polymerization/Depolymerization Assay Kit (Fluorometric) (BN00695)

(Catalog # BN00695; 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. Assay Genie'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	BN00695	Cap Code	Part Number
Buffer G	20 ml	WM	BN00695-1
Buffer P (10X)	1.5 ml	Clear	BN00695-2
Labeled Rabbit Muscle Actin	4 vials	Green	BN00695-3
ATP (100 mM)	2 X 100 µl	Yellow	BN00695-4

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 -20ºC. 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 buffer at -20ºC. 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 -20º 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 µl	70 µl	60 µl
Actin	20 µl	20 µl	20 µl
Test Sample	-	-	10 µl

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 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 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 µl	60 µl
Supp. Buffer P (10X)	10 µl	10 µl
Actin	20 µl	20 µl

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 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 room temperature. 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)

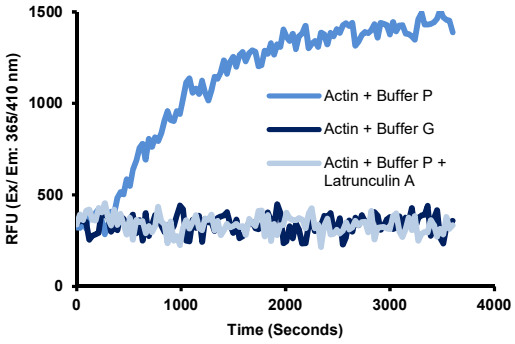
ΔRFU_p = Generated fluorescence of Actin in presence of Buffer P (polymeric actin)

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)

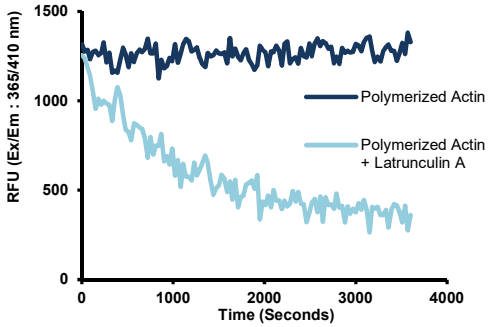


Figure: (a) **Actin Polymerizarion**: Actin Polymerization is induced by Buffer P. The process is inhibited by Latrunculin A (23 µM) (b) **Actin Depolymerization:** Polymerized Actin is depolymerized by Latrunculin A (23 µM). Assays were performed following the kit protocol.
Note: Latrunculin A is Actin 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).

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