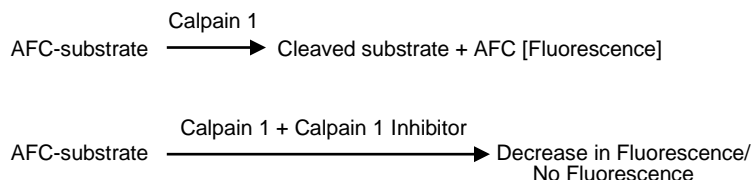


Human Calpain 1 Inhibitor Screening Kit (Fluorometric) rev. 12/18

(Catalog # K244-100; 100 assays; Store kit at -80°C)

I. Introduction:

Calpain 1 is calcium-dependent, non-lysosomal cysteine protease with low calcium requirement. It is expressed ubiquitously and in the brain, mainly localized to the cell body and dendrites of neurons and to a lesser extent in axons and glial cells. Hyperactivation of calpains is implicated in a number of pathologies associated with altered calcium homeostasis such as Alzheimer's disease as well as secondary degeneration resulting from acute cellular stress following cerebral (neuronal) ischemia, traumatic brain injury and spinal cord injury. BioVision's human calpain 1 inhibitor screening kit is based on the detection of cleavage of calpain substrate Ac-LLY-AFC. Ac-LLY-AFC possess very weak fluorescence; upon cleavage of the substrate by calpain 1, free AFC can be quantified fluorometrically (Ex/Em = 400/505 nm). In the presence of potent Calpain 1 inhibitor, the hydrolyzation of substrate will be impeded. The kit provides a rapid, simple, sensitive, and reliable test suitable for high throughput screening of Calpain 1 inhibitors. Inhibitor control (Calpain inhibitor 1, ALLN) is included to compare the efficacy of test inhibitors.



II. Application:

- Screening human Calpain 1 inhibitors.

III. Kit Contents:

Components	K244-100	Cap Code	Part Number
Calpain 1 Assay Buffer	25 ml	WM	K244-100-1
Calpain 1 Substrate	0.2 ml	Red	K244-100-2
Calpain 1 Enzyme	0.2 ml	Green	K244-100-3
Inhibitor Control (Calpain Inhibitor 1, ALLN)[1 μM]	0.5 ml	Blue	K244-100-4

IV. User Supplied Reagents and Equipments:

- 96-well, opaque black plates are preferred for fluorescence reading
- Fluorescence microplate reader

V. Storage and Handling:

Store kit at -80°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials before opening. Read the entire protocol before performing the assay.

VI. Reagent preparation:

- **Calpain 1 Enzyme:** Aliquot and store at -80°C. Avoid repeated freeze/thaw cycles. Use within one month.
- **Calpain 1 Substrate:** Aliquot and store at -80°C. Avoid repeated freeze/thaw.
- **Inhibitor Control (Calpain Inhibitor 1, ALLN):** Aliquot and store at -80°C. Avoid repeated freeze/thaw.

VII. Calpain 1 Inhibitor Screening Assay Protocol:

1. **Enzyme Preparation:** For each well, prepare 25 μl Calpain 1 enzyme solution.

23 μl Calpain 1 Assay Buffer
2 μl Calpain 1 enzyme

2. **Screen compounds, inhibitor control and blank control Preparations:**

Dissolve candidate inhibitors into proper solvent. Dilute to 2X the desired test concentration with Assay Buffer. Add 50 μl diluted test inhibitors, Inhibitor Control or Assay Buffer into Calpain 1 enzyme wells as sample screen [S], Inhibitor Control (Calpain Inhibitor 1, ALLN), or Enzyme Control [EC] (no inhibitor). Mix well, and incubate for 5 minutes at 37°C.

3. **Substrate preparation:** For each well, prepare 25 μl substrate solution.

23 μl Calpain 1 Assay Buffer
2 μl Calpain 1 Substrate

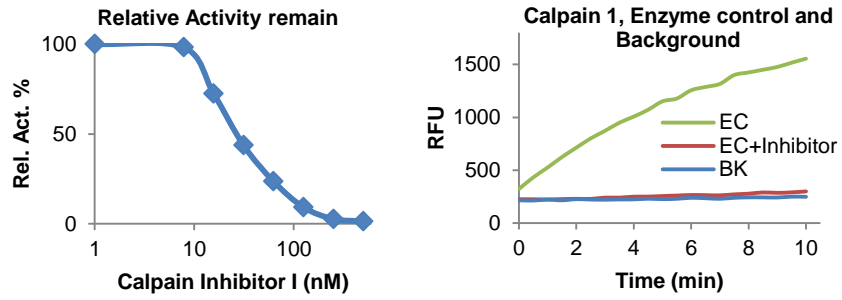
Mix, add 25 μl substrate solution into each well. Mix well.

4. **Measurement:** Read fluorescence (R₁) at Ex/Em = 400/505 nm. Incubate the reaction at 37°C for 10 min, protected from light and measure again fluorescence (R₂) at Ex/Em = 400/505 nm.

5. **Calculation:** The RFU of fluorescence generated by hydrolyzation of substrate is ΔRFU = R₂ – R₁. Set the ΔRFU of Enzyme Control [EC] as 100%, and calculate the relative % inhibition of the test inhibitors as:

$$\% \text{ Inhibition} = \frac{\Delta \text{RFU of EC} - \Delta \text{RFU of S}}{\Delta \text{RFU of EC}} \times 100\%$$

Note: It is recommended to read kinetically to choose the R_1 and R_2 at linear range. We suggest reading the fluorescence every 30 seconds.



VIII. Related Products:

Active Human Calpain 1
 Calpain Activity Assay Kit
 Calpain 1 blocking peptide
 Calpain-2 blocking peptide
 Calpain Inhibitor Z-LLY-FMK
 Suc-Leu-Leu-Val-Tyr-AMC

Calpain Inhibitor 1, ALLN
 Calpain Inhibitor II, ALLM
 Calpain 1 Antibody
 Calpain-2 Antibody
 PD 150,606

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