

Calpain Activity Assay Kit

(Catalog #K240-100; 100 assays; Store kit at -70°C)

I. Introduction:

Activation of calpain is involved in many forms of physiological and pathological processes (e.g., apoptosis). Calpain activation requires cell membrane and Ca^{2+} , and activated calpain is released into cytosol. The **Calpain Activity Assay Kit** provides optimized buffers and reagents for a convenient measurement of calpain activity. The Extraction Buffer provided with the kit specifically extracts cytosolic proteins without contaminations of cell membrane and lysosome proteases. The Extraction Buffer also prevents auto-activation of calpain during the extraction procedure. Thus, the kit detects only activated calpain in cytosol upon treatment of cells with inducers (e.g., chemicals or drugs). The fluorometric assay is based on the detection of cleavage of calpain substrate Ac-LLY-AFC. Ac-LLY-AFC emits blue light ($\lambda_{\text{max}} = 400 \text{ nm}$); upon cleavage of the substrate by calpain, free AFC emits a yellow-green fluorescence ($\lambda_{\text{max}} = 505 \text{ nm}$), which can be quantified using a fluorometer or a fluorescence plate reader. Comparison of the fluorescence intensity from a treated sample with a normal control allows determination of the changes in calpain activity.

II. Kit Contents:

Components	K240-100	Cap Code
	100 assays	Cap Color
Extraction Buffer	25 ml	WM
10X Reaction Buffer	1.5 ml	Clear
Calpain Substrate Ac-LLY-AFC	0.5 ml	Amber
Active Calpain I (Positive Control)	10 μl	Green
Calpain Inhibitor Z-LLY-FMK	10 μl	Orange

III. Calpain Assay Procedure:

- Treat cells by desired methods. Concurrently incubate a control culture without treatment.
- Count cells and pellet $\sim 1\text{-}2 \times 10^6$ cells by centrifugation.
- Resuspend cells in 100 μl Extraction Buffer and incubate samples on ice for 20 minutes. Gently mix the samples by tapping several times during incubation.
- Centrifuge for 1 min in a microcentrifuge (10,000 g) and transfer supernatant to a fresh tube and put on ice. Assay protein concentration.
- Dilute the cell lysate ($\sim 50\text{-}200 \mu\text{g}$) to 85 μl of Extraction Buffer.
For positive control, add 1-2 μl Active Calpain to 85 μl of Extraction Buffer.
For negative control, use untreated cell lysate or add 1 μl Calpain Inhibitor to the treated cell lysate.
- Add 10 μl of 10X Reaction Buffer and 5 μl of Calpain Substrate to each assay.
- Incubate at 37°C for 1 hour in the dark.
- Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. For a plate reading set-up, transfer the samples to a 96-well plate.

- The changes in calpain activity can be determined by comparing results of treated samples and negative control. Alternatively, the activity can be expressed as Relative Fluorescent Unit (RFU) per milligram protein of each sample.

IV. Storage and Stability:

- Store kit at -70°C (Store Extraction Buffer and 10X Reaction Buffer at 4°C after opening). All reagents are stable for 6 months under proper storage conditions.

VI. Related Products:

Apoptosis Detection Kits & Reagents

- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents

Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

Cell Proliferation & Senescence

- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

Cell Damage & Repair

- HDAC Fluorometric & Colorimetric Assays & Drug Discovery Kits
- HAT Colorimetric Assay Kit & Reagents
- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Fluorometric & Colorimetric Assay Kits

Signal Transduction

- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

Adipocyte & Lipid Transfer

- Recombinant Adiponectin, Survivin, & Leptin
- CETP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

Molecular Biology & Reporter Assays

- siRNA Vectors
- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits
- 5 Minutes DNA Ligation Kit
- 20 Minutes Gel Staining/Destaining Kit