3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Calpain Activity Fluorometric Assay Kit

Catalog Number **MAK228**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description

Activation of calpain is involved in many physiological and pathological processes (e.g., apoptosis). Calpain activation requires cell membrane and Ca^{2+}, and activated calpain is released into the 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 contamination with 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 the cytosol upon treatment of cells with inducers (e.g., chemicals or drugs).

The fluorometric assay is based on the cleavage of the calpain substrate, Ac-LLY-AFC. The Ac-LLY-AFC substrate emits blue light (λ_{max} = 400 nm); upon cleavage by calpain, free AFC emits a yellow-green fluorescence (λ_{max} = 505 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.

Components

The kit is sufficient for 100 assays in 96 well plates.

Extraction Buffer Catalog Number MAK228A	25 mL
10× Reaction Buffer Catalog Number MAK228B	1.5 mL
Calpain Substrate Ac-LLY-AFC Catalog Number MAK228C	0.5 mL
Active Calpain I (Positive Control) Catalog Number MAK228D	10 µL
Calpain Inhibitor Z-LLY-FMK Catalog Number MAK228E	10 µL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the kit at –70 °C. Store the Extraction Buffer and 10× Reaction Buffer at 2–8 °C after opening.

Procedure
All samples and standards should be run in duplicate.

Sample Preparation
Treat cells by desired methods. Concurrently incubate a control culture without treatment. Count cells and pellet 1–2 × 10^6 cells by centrifugation. Resuspend cells in 100 µL of Extraction Buffer and incubate samples on ice for 20 minutes. Gently mix the samples by tapping several times during incubation. Centrifuge for 1 minute in a microcentrifuge (10,000 × *g*) and transfer supernatant to a fresh tube and put on ice.

Assay protein concentration.
Note: Due to the high reducing agent content in the Extraction Buffer, dilute supernatant ~10-fold, then use a Coomassie-based protein assay.

Dilute the cell lysate (50–200 µg) to 85 µL with Extraction Buffer.

Assay Reaction
For a positive control, add 1–2 µL of Active Calpain (Catalog Number MAK228D) to 85 µL of Extraction Buffer. For a negative control, use untreated cell lysate or add 1 µL of Calpain Inhibitor (Catalog Number MAK228E) to the treated cell lysate. Add 10 µL of 10× 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.

Results
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.

A new standard curve must be set up each time the assay is run.

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Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range