

Calpain Activity Assay Kit ab65308

★★★★★ [1 Abreviews](#) [74 References](#) [3 Images](#)

Overview

Product name	Calpain Activity Assay Kit
Detection method	Fluorescent
Sample type	Tissue Extracts, Cell Lysate
Assay type	Quantitative
Assay time	2h 00m
Species reactivity	Reacts with: Mammals, Other species
Product overview	Calpain Activity Assay Kit ab65308 provides optimized buffers and reagents for a convenient measurement of calpain activity.

The extraction buffer included in the kit specifically extracts cytosolic proteins without contamination by cell membrane and lysosome proteases. It also prevents auto-activation of calpain during the extraction procedure. Thus, the kit detects only activated calpain within the cytosol.

The calpain activity assay protocol 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.

Calpain activity assay protocol summary:

- add samples and positive and negative controls to wells
- add reaction buffer and calpain substrate
- incubate for 60 min
- analyze with a microplate reader

Notes This product is manufactured by BioVision, an Abcam company and was previously called K240 Calpain Activity Fluorometric Assay Kit. K240-100 is the same size as the 100 test size of ab65308.

If additional Ac-LLY-AFC substrate is needed, it can be purchased separately as [ab171379](#).

Platform Microplate reader

Properties

Storage instructions

Store at -80°C. Please refer to protocols.

Components	100 tests
10X Reaction Buffer	1 x 1.5ml
Calpain I Positive Control	1 x 10µl
Calpain Inhibitor	1 x 10µl
Calpain Substrate	1 x 500µl
Extraction Buffer III	1 x 25ml

Function

Calcium-regulated non-lysosomal thiol-protease which catalyze limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction.

Tissue specificity

Ubiquitous.

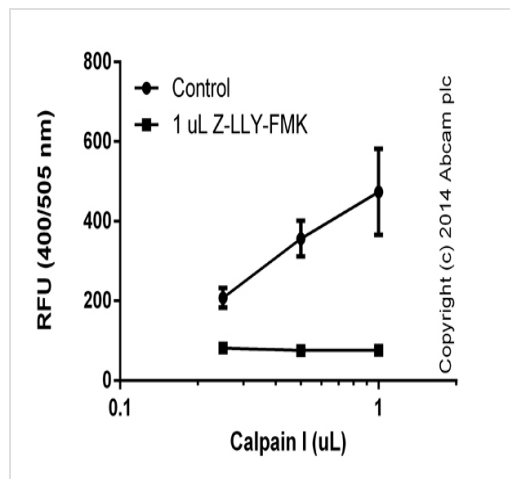
Sequence similarities

Belongs to the peptidase C2 family.
Contains 1 calpain catalytic domain.
Contains 4 EF-hand domains.

Cellular localization

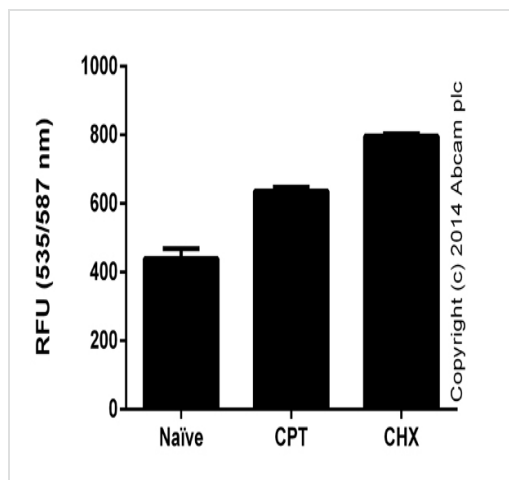
Cytoplasm. Cell membrane. Translocates to the plasma membrane upon Ca(2+) binding.

Images



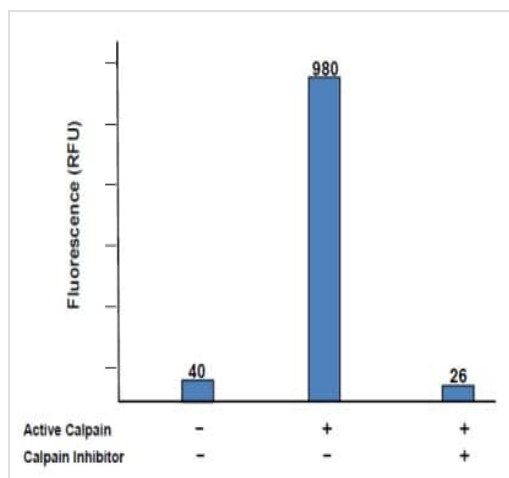
Different amounts of positive control (Calpain I) treated with 1 µL of inhibitor (Z-LLY-FMK), background signal subtracted, duplicates; +/- SD.

Functional assays: Calpain Activity Assay Kit
(ab65308)



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10e7 Jurkat cells (in 10 mL) were cultured in the absence or presence of 10 μ M Camptothecin (CPT) ([ab120115](#)) or 10 μ g/mL Cycloheximide (CHX) ([ab120093](#)) for 4 hours. Pelleted cells were lysed in 0.5 mL of Extraction Buffer and tested directly. Background signal subtracted, duplicates; \pm SD.



Functional Studies - Calpain Activity Assay Kit
(ab65308)

Typical Data for ab65308: Active Calpain (1 μ g) was incubated at 37 $^{\circ}$ C for one hour using the Calpain Substrate with or without 20 μ M Calpain Inhibitor.

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