Calpain Activity Assay Kit ab65308

**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 (λmax = 400 nm); upon cleavage of the substrate by calpain, free AFC emits a yellow-green fluorescence (λmax = 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.

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**

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.
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.

Functional assays: Calpain Activity Assay Kit (ab65308)

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

10e7 Jurkat cells (in 10 mL) were cultured in the absence or presence of 10 μM Camptotheacin (CPT) (ab120115) or 10 μg/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; +/- SD.

Components

<table>
<thead>
<tr>
<th>Components</th>
<th>Identifier</th>
<th>100 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Reaction Buffer</td>
<td>Clear</td>
<td>1 x 1.5ml</td>
</tr>
<tr>
<td>Active Calpain I (Positive Control)</td>
<td>Green</td>
<td>1 x 10μl</td>
</tr>
<tr>
<td>Calpain Inhibitor Z-LLY-FMK</td>
<td>Orange</td>
<td>1 x 10μl</td>
</tr>
<tr>
<td>Calpain Substrate Ac-LLY-AFC</td>
<td>Amber</td>
<td>1 x 500μl</td>
</tr>
<tr>
<td>Extraction Buffer</td>
<td>WM</td>
<td>1 x 25ml</td>
</tr>
</tbody>
</table>

Images

Functional assays: Calpain Activity Assay Kit (ab65308)
Typical Data for ab65308: Active Calpain (1 µg) was incubated at 37 °C for one hour using the Calpain Substrate with or without 20 µM Calpain Inhibitor.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors