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
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.

<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:

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 Camptothecin (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.
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.

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