Human Pro Caspase-3 ELISA Kit ab192146

Overview

Product name: Human Pro Caspase-3 ELISA Kit
Detection method: Colorimetric

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jurkat</td>
<td>5</td>
<td>5.2%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jurkat</td>
<td>3</td>
<td>4.9%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sample type: Cell culture extracts, Tissue Extracts, Cell Lysate
Assay type: Sandwich (quantitative)
Sensitivity: 3 pg/ml
Range: 0.156 ng/ml - 10 ng/ml
Recovery: Sample specific recovery

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Average %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell culture media</td>
<td>73.3</td>
<td>70.7% - 76.1%</td>
</tr>
</tbody>
</table>

Assay time: 1h 30m
Assay duration: One step assay
Species reactivity: Reacts with: Human

Product overview:
Pro Caspase-3 in vitro SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Pro Caspase-3 protein in human cell and tissue extract samples. (UniprotID: P42574)

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter
conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

**General sensitivity:**

3 pg/mL for measurement of lysates prepared by direct in well lysis.

11 pg/mL for measurement of extracts prepared from cell pellets and tissue homogenates.

**Notes**

Caspase-3 is a cysteine protease involved in the activation cascade of caspases responsible for apoptosis execution. At the onset of apoptosis Caspase-3 proteolytically cleaves poly (ADP-ribose) polymerase (PARP) at Asp216-Gly217 bond. Caspase-3 cleaves and activates sterol regulatory element binding proteins (SREBPs) between the basic helix-loop-helix leucine zipper domain and the membrane attachment domain. Caspase-3 cleaves and activates caspase-6, -7 and -9. Caspase-3 is involved in the cleavage of huntingtin. Caspase-3 is a cytoplasmic protein highly expressed in lung, spleen, heart, liver and kidney. Moderate levels of Caspase-3 are in brain and skeletal muscle, and low levels in testis. Also Caspase-3 is found in many cell lines, highest expression in cells of the immune system. Caspase-3 is expressed in an inactive pro-form (pro Caspase-3). In apoptosis, the pro Caspase-3 is activated by proteolytic cleavages at Asp28-Ser29 and Asp175-Ser176 bond catalyzed by granzyme B, caspase-6, caspase-8, caspase-9 and caspase-10 generating two active subunits. Thus the pro-form and the active form are useful biomarkers of apoptosis. Active Caspase-3 is a heterotetramer that consists of two anti-parallel arranged heterodimers, each one formed by a 17 kDa (p17) and a 12 kDa (p12) subunit. Additional processing of the propeptide is likely due to the autocatalytic activity of the activated protease. Active heterodimers between the small subunit of caspase-7 protease and the large subunit of caspase-3 also occur and vice versa. Caspase-3 is S-nitrosylated on its catalytic site cysteine in unstimulated human cell lines and denitrosylated upon activation of the Fas apoptotic pathway, associated with an increase in intracellular caspase activity. Fas therefore activates caspase-3 not only by inducing the cleavage of the caspase zymogen to its active subunits, but also by stimulating the denitrosylation of its active site thiol.

**Tested applications**

Suitable for: Sandwich ELISA

**Platform**

Microplate (12 x 8 well strips)

**Properties**

**Storage instructions**

Store at +4°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>1 x 96 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Pro Caspase-3 Capture Antibody</td>
<td>1 x 600µl</td>
</tr>
<tr>
<td>10X Pro Caspase-3 Detector Antibody</td>
<td>1 x 600µl</td>
</tr>
<tr>
<td>10X Wash Buffer PT (ab206977)</td>
<td>1 x 20ml</td>
</tr>
</tbody>
</table>
Relevance

- Involved in the activation cascade of caspases responsible for apoptosis execution. At the onset of apoptosis it proteolytically cleaves poly(ADP-ribose) polymerase (PARP) at a '216-Asp-|-Gly-217' bond.
- Cleaves and activates sterol regulatory element binding proteins (SREBPs) between the basic helix-loop-helix leucine zipper domain and the membrane attachment domain.
- Cleaves and activates caspase-6, -7 and -9.
- Involved in the cleavage of huntingtin.
- Triggers cell adhesion in sympathetic neurons through RET cleavage.

Cellular localization

- Cytoplasm.

Applications

Our Abpromise guarantee covers the use of ab192146 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandwich ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>

Images
Example of Pro Caspase-3 standard curve for measurement of lysates prepared from cells in media (RPMI1640 supplemented with 10% fetal Bovine serum) by direct in well lysis.

Background-subtracted data values (mean +/- SD) are graphed.

Example of Pro Caspase-3 standard curve for measurement of extracts prepared from cell pellets and tissue extracts.

Background-subtracted data values (mean +/- SD) are graphed.

Titration of Jurkat cell extracts within the working range of the assay.

Background-subtracted data values (mean +/- SD, n = 2) are graphed.
Jurkat cell lysates corresponding to 2x10^6 cells/mL or 1x10^6 cells/mL were prepared as previously described. The concentrations of Pro Caspase 3 were interpolated from data values shown in the previous figure using Pro Caspase 3 standard curve and graphed in ng of Pro Caspase-3 per 10^6 cells. Staurosporine IC_{50} determined from the interpolated data values were 0.70 µM and 0.56 µM using, respectively, lysates of 2x10^6 cells/mL and 1x10^6 cells/mL.

Jurkat cell lysates corresponding to 2x10^6 cells/mL or 1x10^6 cells/mL were prepared by direct in-well lysis (without media removal) from cells grown in RPMI1640 media supplemented with 10% FBS and treated for 4 hours with variable doses of staurosporine in a 96-well plate. Background-subtracted data values (mean +/- SD, n=3) are graphed. Staurosporine IC_{50} determined from background-subtracted data values were 0.75 µM and 0.57 µM using, respectively, lysates of 2x10^6 cells/mL and 1x10^6 cells/mL.

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