Overview

**Product name**
Caspase-3 Immunoassay Kit (Fluorometric)

**Detection method**
Fluorescent

**Sample type**
Cell Lysate

**Assay type**
Quantitative

**Product overview**
The Caspase 3 Immunoassay Kit (Fluorometric) (ab234045) provides an effective immunosorbent enzyme assay for specific, quantitative detection of caspase 3 activity in microtiter plates. The assay utilizes caspase 3 polyclonal antibody to capture activated caspase 3 from cell lysates. Caspase substrate DEVD-AFC is then added and is cleaved proportionally to the amount of activated caspase 3 in the cell lysate. The cleavage generates free AFC which can be analyzed fluorometrically (Ex/Em = 400 nm/505 nm) using a fluorescence plate reader. The assay ensures absolute specific detection of caspase 3. Other known caspases and non-specific proteases are not detected.

**Notes**
Activation of caspase 3 plays a key role in initiation of cellular events during apoptosis.

**Platform**
Microplate reader

Properties

**Storage instructions**
Store at -20°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>100 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Lysis Buffer</td>
<td>1 x 25ml</td>
</tr>
<tr>
<td>Coating Buffer</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>Anti-Caspase 3 Antibody (20X)</td>
<td>1 x 0.5ml</td>
</tr>
<tr>
<td>Blocking Buffer</td>
<td>1 x 15ml</td>
</tr>
<tr>
<td>Incubation Buffer</td>
<td>1 x 100ml</td>
</tr>
<tr>
<td>DTT (1M)</td>
<td>1 x 400µl</td>
</tr>
<tr>
<td>DEVD-AFC Substrate (1 mM)</td>
<td>1 x 500µl</td>
</tr>
<tr>
<td>Positive Control (rh-Caspase 3)</td>
<td>1 x 10 units</td>
</tr>
</tbody>
</table>
Function
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.

Tissue specificity
Highly expressed in lung, spleen, heart, liver and kidney. Moderate levels in brain and skeletal muscle, and low in testis. Also found in many cell lines, highest expression in cells of the immune system.

Sequence similarities
Belongs to the peptidase C14A family.

Post-translational modifications
Cleavage by granzyme B, caspase-6, caspase-8 and caspase-10 generates the two active subunits. Additional processing of the propeptides 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. 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.

Cellular localization
Cytoplasm.

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