Human Cleaved PARP1 ELISA Kit ab174441

**Overview**

**Product name**  Human Cleaved PARP1 ELISA Kit  
**Detection method**  Colorimetric

**Precision**

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intra-assay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>5</td>
<td>6.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inter-assay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>3</td>
<td>8.5%</td>
<td></td>
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</tr>
</tbody>
</table>

**Sample type**  Cell culture extracts  
**Assay type**  Sandwich (quantitative)  
**Sensitivity**  5.8 µg/ml  
**Range**  7.8 µg/ml - 500 µg/ml  
**Recovery**

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Average %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell culture media</td>
<td>59</td>
<td>43% - 73%</td>
</tr>
<tr>
<td>Fetal Bovine Serum</td>
<td>65</td>
<td>52% - 85%</td>
</tr>
<tr>
<td>Bovine Serum Albumin</td>
<td>75</td>
<td>57% - 94%</td>
</tr>
</tbody>
</table>

**Assay time**  1h 30m  
**Assay duration**  One step assay  
**Species reactivity**  Reacts with: Human  
**Product overview**  Abcam’s cleaved PARP1 in vitro SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay)
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.

Notes

PARP1 is a 113 kDa nuclear DNA-repair enzyme that transfers ADP-ribose units from NAD+ to variety of nuclear proteins including topoisomerases, histones and PARP1 itself. Via poly ADP ribosylation, PARP1 is responsible for regulation of cellular homeostasis including cellular repair, transcription and replication of DNA, cytoskeletal organization and protein degradation. In response to DNA damage, PARP1 activity is increased upon binding to DNA strand nicks and breaks. Excessive DNA damage leads to generation of large branched ADP-ribose polymers and activation of a unique cell death program.

During apoptosis, PARP1 is cleaved by activated caspase-3 between Asp214 and Gly215, resulting in the formation of an N-terminal 24 kDa fragment containing most of the DNA binding domain and a C-terminal 89 kDa fragment containing the catalytic domain. The proteolysis of PARP1 through this cleavage renders the enzyme inactive and this further facilitates apoptotic cell death. Thus the presence of 89 kDa PARP1 fragment is considered to be a very reliable biomarker of apoptosis.

Tested applications

Suitable for: Sandwich ELISA

Platform

Microplate

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 Cleaved PARP1 Detector Antibody</td>
<td>1 x 600µl</td>
</tr>
<tr>
<td>10X Wash Buffer PT (ab206977)</td>
<td>1 x 20ml</td>
</tr>
<tr>
<td>50X Cell Extraction Enhancer Solution (ab193971)</td>
<td>1 x 1ml</td>
</tr>
<tr>
<td>5X Cell Extraction Buffer PTR (ab193970)</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>Antibody Diluent 5B</td>
<td>1 x 6ml</td>
</tr>
<tr>
<td>Cleaved PARP1 Capture Antibody</td>
<td>1 x 2 vials</td>
</tr>
</tbody>
</table>
Function
Involved in the base excision repair (BER) pathway, by catalyzing the poly(ADP-ribosyl)ation of a limited number of acceptor proteins involved in chromatin architecture and in DNA metabolism. This modification follows DNA damages and appears as an obligatory step in a detection/signaling pathway leading to the reparation of DNA strand breaks. Mediates the poly(ADP-ribosyl)ation of APLF and CHFR. Positively regulates the transcription of MTUS1 and negatively regulates the transcription of MTUS2/TIP150. With EEF1A1 and TXK, forms a complex that acts as a T-helper 1 (Th1) cell-specific transcription factor and binds the promoter of IFN-gamma to directly regulate its transcription, and is thus involved importantly in Th1 cytokine production. Required for PARP9 and DTX3L recruitment to DNA damage sites. PARP1-dependent PARP9-DTX3L-mediated ubiquitination promotes the rapid and specific recruitment of 53BP1/TP53BP1, UIMC1/RAP80, and BRCA1 to DNA damage sites.

Sequence similarities
Contains 1 BRCT domain.
Contains 1 PARP alpha-helical domain.
Contains 1 PARP catalytic domain.
Contains 2 PARP-type zinc fingers.

Post-translational modifications
Phosphorylated by PRKDC and TXK.
Poly-ADP-ribosylated by PARP2. Poly-ADP-ribosylation mediates the recruitment of CHD1L to DNA damage sites.
S-nitrosylated, leading to inhibit transcription regulation activity.

Cellular localization
Nucleus. Nucleus, nucleolus. Localizes at sites of DNA damage.

Applications
Our Abpromise guarantee covers the use of ab174441 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>Use at an assay dependent concentration.</td>
<td></td>
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</tbody>
</table>
Example of HeLa Staurosporine (ab120056) treated cell lysate titration.

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

Untreated and staurosporine (ab120056) treated HeLa and Jurkat lysates were prepared in 1X Cell Extraction Buffer PTR and tested using the Cleaved PARP1 SimpleStepELISA. Raw OD 450 nm values are shown for 500 µg/mL lysate loads.

HeLa and Jurkat cells were treated with 1 µM Staurosporine (STS) for 4 hours in complete cell culture media to induce apoptosis and cleaved PARP1 protein.

Example of IC50 determination.

HeLa cells were treated with a dose titration of Staurosporine for 4 hours in complete media. Cells were cultured and treated in a 96-well cell culture microtiter plate. Lysates were prepared by direct in-well lysis without media removal: 2X Cell Extraction Buffer PTR was added to an equal volume of media and then resulting lysate was used directly in the Cleaved PARP1 SimpleStep™ ELISA assay. Raw values for triplicate measurements are plotted. The calculated IC50 is 0.77 µM.
20 µg of HeLa extracts that were untreated or treated for 4 hours with 1 µM Staurosporine were analyzed by western blot. The GAPDH blot is included to show the relative loads of each lysate. In the HeLa cell line, Staurosporine treatment is required to detect cleaved PARP1 protein, as observed in the SimpleStep ELISA (Figure 2).

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