



ab119690

**Cleaved PARP Human
ELISA Kit**

Instructions for Use

For the quantitative measurement of human cleaved PARP protein.

This product is for research use only and is not intended for diagnostic use.

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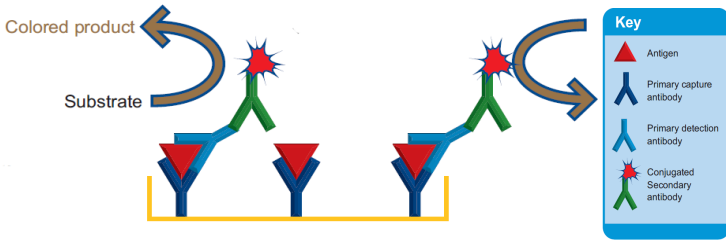
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1. Introduction

Principle: ab119690 Cleaved PARP Human ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of 89 kDa fragment of Human PARP-1 in cell and tissue lysates, including lysates of adherent and suspension cells grown in 96-well microplate format and lysed directly in culture media. The assay employs an antibody specific for the 89 kDa fragment of Human PARP-1 coated onto well plate strips. This antibody reacts with the N-terminal end formed by the cleavage adjacent to Asp214; it thus recognizes the apoptosis-specific 89 kDa catalytic domain fragment, but it does not recognize the full-length PARP-1 or the 24 kDa DNA binding domain fragment. Standards and samples are pipetted into the wells and analyte present in the sample is bound to the wells by the immobilized antibody. The wells are washed and an anti- PARP-1 primary detector antibody is added. After washing away unbound primary detector antibody, HRP-label specific for the primary detector antibody is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of analyte bound. The developing blue color is measured at 600 nm. Optionally the reaction can be stopped by adding hydrochloric acid which changes the color from blue to yellow and the intensity can be measured at 450 nm.

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Background: PARP-1 is a 113 kDa nuclear DNA-repair enzyme that transfers ADP-ribose units from NAD⁺ to variety of nuclear proteins including topoisomerases, histones and PARP-1 itself. Via poly ADP ribosylation, PARP-1 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, PARP-1 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, PARP-1 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 PARP-1 through this cleavage renders the enzyme inactive and this further facilitates apoptotic cell death. Thus the presence of 89 kDa PARP-1 fragment is considered to be a very reliable biomarker of apoptosis.

2. Assay Summary

Bring all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



Add 50 μ L standard or sample to each well used. Incubate 2 hours at room temperature.



Aspirate and wash each well two times. Add 50 μ L prepared 1X Detector Antibody to each well. Incubate 1 hour at room temperature.



Aspirate and wash each well two times. Add 50 μ L prepared 1X HRP Label. Incubate 1 hour at room temperature.



Aspirate and wash each well three times. Add 50 μ L TMB Development Solution to each well. Immediately begin recording the color development for 15 minutes at 600 nm. Alternatively add a Stop solution at a user-defined time and read at 450 nm.

3. Kit Contents

Item	Quantity
2X Extraction Buffer	15 mL
20X Buffer	20 mL
10X Blocking Buffer	6 mL
PARP Microplate (12 x 8 antibody coated well strips)	96 Wells
10X Detector Antibody	0.7 mL
10X HRP Label	1 mL
TMB Development Solution	6 mL

4. Storage and Handling

Store all components at 4 °C. This kit is stable for at least 6 months from receipt. After reconstitution the standard should be stored at -80 °C. Unused microplate strips should be returned to the pouch containing the desiccant and resealed.

5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 600 nm (or 450 nm after addition of Stop solution - not supplied).
- Method for determining protein concentration (BCA assay recommended).
- Deionized water
- Multi and single channel pipettes
- PBS (1.4 mM KH_2PO_4 , 8 mM Na_2HPO_4 , 140 mM NaCl, 2.7 mM KCl, pH 7.3)
- Tubes for standard dilution
- Stop solution (optional) – 1N hydrochloric acid
- Optional plate shaker for all incubation steps

6. Preparation of Reagents

- 6.1. Equilibrate all reagents to room temperature (18-25°C) before use.
- 6.2. If preparing lysates from cell pellets prepare 1X Extraction Buffer by adding 15 mL 2X Extraction Buffer to 15 mL nanopure water. Mix gently and thoroughly.
- 6.3. Prepare 1X Wash Buffer by adding 20 mL 20X Buffer to 380 mL nanopure water. Mix gently and thoroughly.
- 6.4. Prepare 1X Incubation Buffer by adding 6 mL 10X Blocking Buffer to 54 mL 1X Wash Buffer. Unused 1X Incubation Buffer may be stored at -20°C for 6 months after performing the ELISA.
- 6.5. Prepare the 1X Detector Antibody by diluting the 10X Detector Antibody 10-fold with 1X Incubation Buffer immediately prior to use. Prepare 0.5 mL for each 8 well strip used.
- 6.6. Prepare the HRP Label by diluting the stock 10X HRP Label 10-fold with 1X Incubation Buffer immediately before use. Prepare 0.5 mL for each 8 well strip used.

7. Sample Preparation

Note: Extraction buffer can be supplemented with phosphatase inhibitors, PMSF and protease inhibitor cocktail prior to use. Supplements should be used according to manufacturer's instructions.

7.1 Preparation of extract from cell pellets

- 7.1.1 Collect non adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. If the adherent cells are detaching, as typical for apoptotic cells, collect both floating cells and remaining adherent cells. Typical centrifugation conditions for cells are 500 x g for 10 min at 4°C.
- 7.1.2 Rinse cells twice with PBS.
- 7.1.3 Solubilize cell pellet at 2×10^7 /mL in 1X Extraction Buffer.
- 7.1.4 Incubate on ice for 20 minutes. Centrifuge at 16,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

7.2 Preparation of extracts from tissue homogenates

- 7.2.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).
- 7.2.2 Suspend the homogenate to 10 mg/mL in PBS.
- 7.2.3 Solubilize the homogenate by adding equal volume of 2X Extraction Buffer to the homogenate.
- 7.2.4 Incubate on ice for 20 minutes. Centrifuge at 16,000 x g for 20 minutes at 4 °C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80 °C. The sample protein concentration in the extract may be quantified using a protein assay.

7.3 Preparation of lysates from cells in media (in-well lysis)

- 7.3.1 Seed cells at the same density into a multi-well plate (e.g. 96-well plate) and treat them as desired.
- 7.3.2 Solubilize the cells by adding equal volume (equal to the volume of culture media) of 2X Extraction Buffer directly to the cells in growth media.

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- 7.3.3 Incubate on ice for 20 minutes. If available use a plate shaker at 300 rpm. Assay the samples immediately by transferring the lysates into the assay Microplate or transfer the lysates into a clean plate and store at -80 °C.

The samples should be diluted to within the working range of the assay in 1:1 mixture of 2X Extraction Buffer and growth media, or 1X Incubation Buffer, as appropriate. As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Data Analysis.

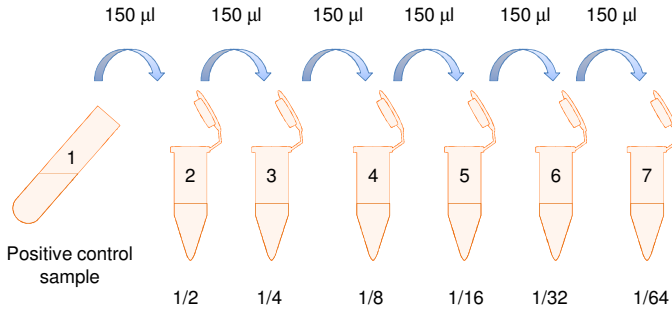
7.4 Preparation of dilution series of positive control sample

Note: It is strongly recommended to prepare a dilution series of a positive control sample. The levels of cleaved PARP are very low in normal healthy cells and tissues. Thus it is recommended to treat cells to induce PARP cleavage and use this sample to prepare the dilution series. For examples of treatment refer to sections 9 and 10. The relative levels of cleaved PARP in other experimental samples can be interpolated from within this positive control sample series.

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- 7.4.1 To prepare serially diluted positive control sample, label six tubes #2-7.
- 7.4.2 Prepare a positive control sample lysate or extract as directed in previous sections (7.1, 7.2 or 7.3). Dilute the positive control sample to an upper concentration limit of the working range of the assay in the same diluent used to dilute other experimental samples. Label this tube #1.
- 7.4.3 Add 150 μL of the diluent to each of tubes #2 through #7.
- 7.4.4 Transfer 150 μL from tube #1 to tube #2. Mix thoroughly. With a fresh pipette tip transfer 150 μL from #2 to #3. Mix thoroughly. Repeat for Tubes #4 through #7. Use the diluent as the zero standard tube labeled #8. Use fresh control sample dilution series for each assay.

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8. Assay Procedure

Equilibrate all reagents and samples to room temperature before use. It is recommended all samples and controls be assayed in duplicate.

- 8.1 Prepare all reagents, working dilutions of controls and samples as directed in the previous sections.
- 8.2 Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and seal.

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- 8.3 Add 50 μL of each sample per well. It is recommended to include a dilution series of a positive control sample (section 7.5), as well as untreated sample. Also include a no material control (diluent) as a zero standard.
- 8.4 Cover/seal the plate and incubate for 2 hours at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.
- 8.5 Aspirate each well and wash, repeat this once more for a total of **two** washes. Wash by aspirating or decanting from wells then dispensing 300 μL of 1X Wash Buffer into each well as described above. Complete removal of liquid at each step is essential to good performance. After the last wash, remove the remaining buffer by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.
- 8.6 Immediately before use prepare sufficient (0.5 mL/strip used) 1X Detector Antibody (step 6.5) in 1X Incubation Buffer. Add 50 μL 1X Detector antibody to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.
- 8.7 Repeat the aspirate/wash procedure above.

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- 8.8 Immediately before use prepare sufficient (0.5 mL/strip used) 1X HRP Label in 1X Incubation Buffer (step 6.6). Add 50 μ L 1X HRP Label to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.
- 8.9 Repeat the aspirate/wash procedure above, however, performing a total of **three** washes.
- 8.10 Add 50 μ L TMB Development Solution to each empty well and immediately record the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nM
Time:	up to 15 min.
Interval:	20 sec. - 1 min.
Shaking:	Shake between readings

Alternative– In place of a kinetic reading, at a **user defined** time record the endpoint OD data at (i) 600 nm or (ii) stop the reaction by adding 50 μ L stop solution (1N HCl) to each well and record the OD at 450 nm.

- 8.11 Analyze the data as described below.

9. Data Analysis

Average the duplicate positive controls readings and plot against their concentrations after subtracting the zero standard reading. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Read relative cleaved PARP concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

TYPICAL STANDARD CURVE - For demonstration only.

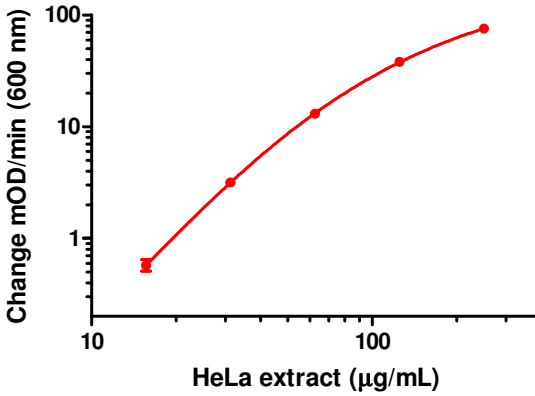


Figure 1. Example standard curve. A dilution series of pellet extracts in Incubation Buffer in the working range of the assay. The lysates were prepared from a pellet of HeLa cells treated with Staurosporine.

TYPICAL EXPERIMENTAL RESULTS - For demonstration only.

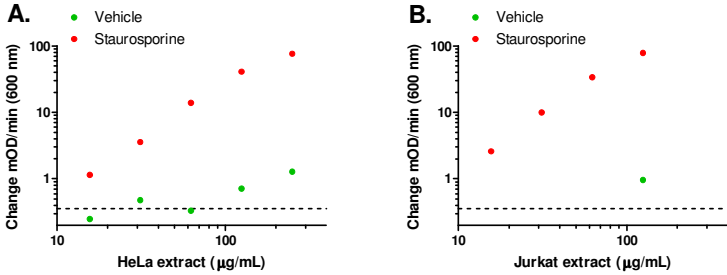


Figure 2. Example experimental analysis of pellet extracts prepared from HeLa (in A) or Jurkat (in B) cells treated with vehicle or Staurosporine. Cell extracts at varying concentrations within the working range of the assay were analyzed. Absolute signals (without zero standard subtraction) are shown. Dashed lines represent signals of zero standard (Incubation Buffer only).

Typical working ranges for pellet extracts (assuming complete PARP cleavage)	
Sample Type	Range
HeLa cell extract, (e.g. HeLa Staurosporine treated)	16 - 250 µg/mL
Jurkat cell extract, (e.g. Jurkat Staurosporine treated)	8 - 125 µg/mL

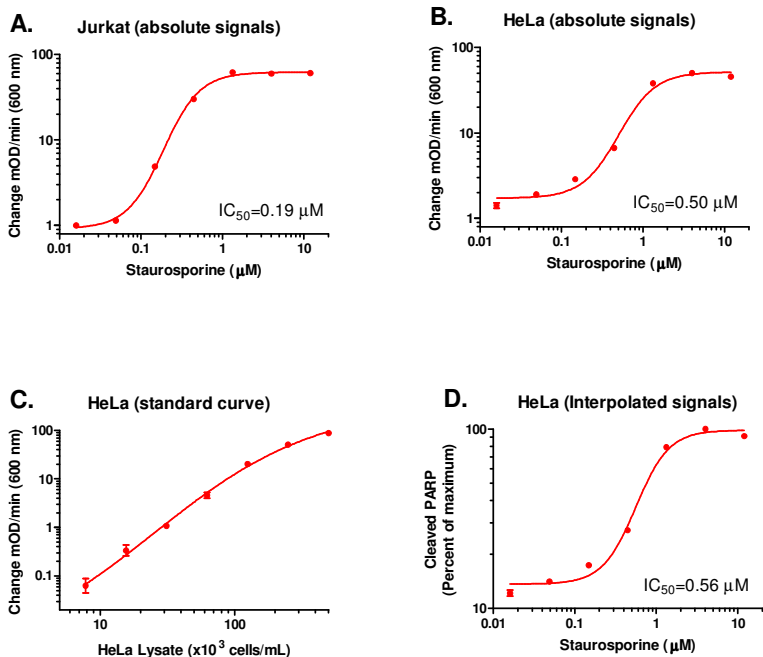


Figure 3. Example of IC_{50} determination. Lysates were prepared by direct in-well lysis without media removal from Jurkat and HeLa cells grown and treated with variable doses of Staurosporine in a 96-well plate. Absolute signals of Jurkat lysates (corresponding to 10×10^5 cells/mL) and HeLa lysates (corresponding to 2.5×10^5 cells/mL) are shown, respectively, in A and B. Standard curve prepared by serial dilution of the in-well lysate (in a 1:1 mixture of 2X Extraction Buffer and Media) of HeLa cells treated with Staurosporine is shown in C. Relative Cleaved PARP concentrations in the Staurosporine-treated HeLa lysates interpolated from standard curve and expressed in percent of maximal signal are shown in D. Note close match of IC_{50} value for HeLa cells determined from absolute and interpolated signals (B and D).

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Typical working ranges for in-well lysis (assuming complete PARP-1 cleavage)	
Sample Type	Range
HeLa cell lysates, (e.g. HeLa Staurosporine treated)	16 - 500 x10 ³ cells/mL
Jurkat cell extract, (e.g. Jurkat Staurosporine treated)	32 – 1000 x10 ³ cells/mL

SENSITIVITY

Determined minimum detectable dose of cleaved PARP (zero dose n=24 + 2 standard deviations) is present in 15 µg/mL extract of Staurosporine-treated HeLa cells (assuming complete PARP-1 cleavage)

LINEARITY OF DILUTION

Linearity of dilution determined by comparing dilution series of extracts prepared from pellets of Staurosporine treated Jurkat cells (starting extract concentration is 125 µg/mL) to extracts prepared from pellets of Staurosporine treated HeLa cells.

Sample Type	% Expected Value
1:1	115
1:2	112
1:4	100
1:8	89
1:16	81

REPRODUCIBILITY

Parameter	CV%
Intra (n=8)	4.9
Inter (n=4)	10.9

RECOVERY

Sample Type	Average Recovery (%)	Range (%)
50% media (10FHGD MEM)	107	105-109
10% bovine serum	103	100-105
10% goat serum	108	107-110
50% Extraction Buffer	88	87-90
25% Extraction Buffer + 25% media	86	82-89

10. Specificity

Species– human reactive. Non-reactive with mouse and rat. Others untested.

Specific marker of apoptosis- reacts with the 89 kDa catalytic domain fragment of PARP-1. Non-reactive with the full-length PARP-1 (113 kDa) or the 24 kDa DNA binding domain fragment.

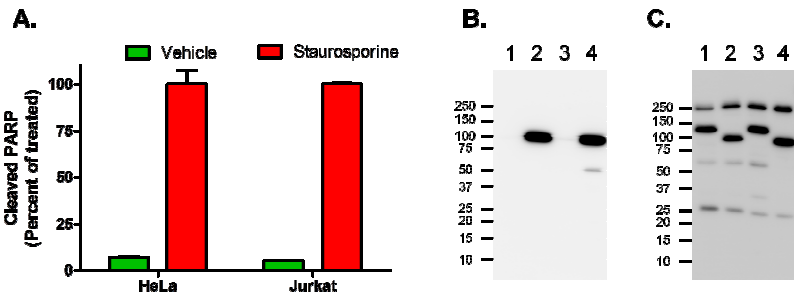


Figure 4. Demonstration of assay specificity by inducing PARP cleavage with Staurosporine. (A) Cleaved PARP ELISA using vehicle or Staurosporine treated HeLa extracts (250 $\mu\text{g}/\text{mL}$) or Jurkat extracts (125 $\mu\text{g}/\text{mL}$). (B) Western blot using the Cleaved PARP ELISA capture antibody (ab110315). (C) Western blot using the Cleaved PARP ELISA detector antibody (ab75607). The same extract preparations of vehicle-treated (lane 1) or Staurosporine-treated (lane 2) HeLa cells, and of vehicle-treated (lane 3) or Staurosporine-treated (lane 4) Jurkat cells were used in both ELISA and Western blot analyses. The data demonstrate that the ELISA kit measures specifically the 89 kDa fragment of PARP-1. Note of complete cleavage of PARP in the Staurosporine-treated HeLa and Jurkat samples (in C).

11. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate Pipetting	Check pipets
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Low cleaved PARP concentration in sample	Use appropriate positive control/treatment to induce apoptosis/PARP cleavage
	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80 °C, all other assay components 4 °C. Keep substrate solution protected from light

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