Product Manual

Poly (ADP-Ribose) ELISA Kit

Catalog Number

AAA11446 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Poly ADP-ribosylation (PAR), the addition of several ADP-ribose groups to proteins, is a post-translational modification in eukaryotic cells that modulates the activity of the modified proteins and their interaction with other ones. This modification is mediated by poly (ADP-ribose) polymerases (PARPs) and ADP-ribosyltransferase 2 (ART2) that use nicotinamide adenine dinucleotide (NAD) as a substrate. ADP-ribosylation can be reversed by PAR-degrading enzymes like poly (ADP-ribose) glycohydrolase (PARG) and ADP-ribosylhydrolase 3 (ARH3) (Figure 1).

Poly ADP-ribosylation is involved in different biological functions like carcinogenesis, differentiation and cell death. Dysregulation of ADP-ribosylation activity has been implicated in the pathogenesis of diseases like cancer, virus infections, and neurodegeneration. One of the most studied roles of ADP-ribosylation is the generation of PAR chains in response to DNA damage. These PAR chains are used to recruit repair proteins and chromatin remodelers. In consequence, DNA repair processes are enhanced. This could be a mechanism for tumors to escape apoptosis. Recently, several inhibitors of PARP family members have shown promise in treating certain cancers, and three drugs are commercially available in the US and Europe, mainly for the treatment of BRCA-mutant ovarian cancer.

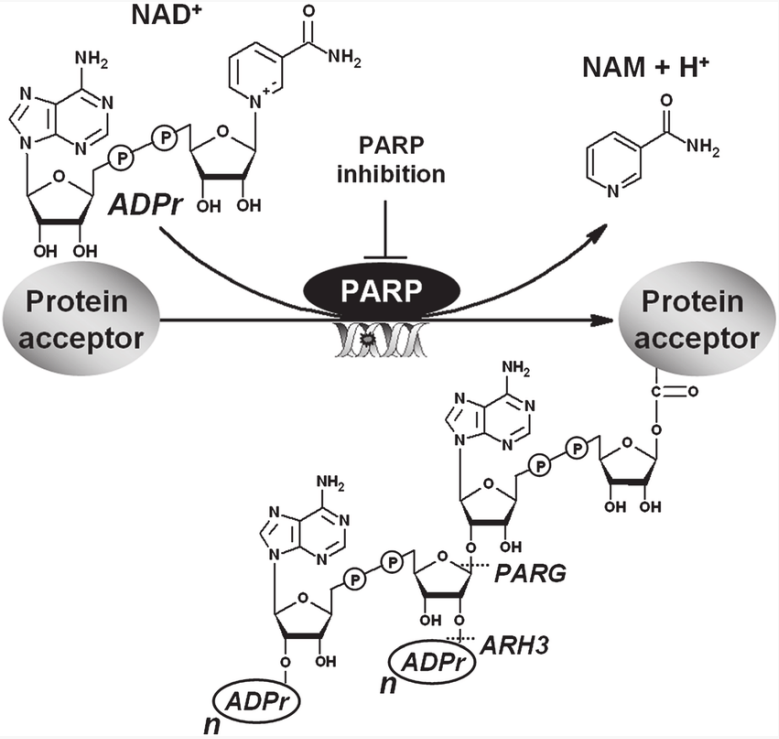

Figure 1. Poly ADP-Ribosylation Reaction. PARP enzymes hydrolyze NAD^+ and transfer ADP-ribose groups (ADPr) to protein acceptors. Several ADPr units linked to each other generate a long negatively charged polymer. These polymers are degraded by PARG and ARH3.

Poly (ADP-ribose) ELISA Kit is an enzyme immunoassay developed for rapid detection and quantitation of PAR levels in cell or tissue samples. The quantity of PAR in an unknown sample

is determined by comparing its absorbance with that of a known standard curve. The kit has a detection sensitivity limit of 20 pM. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

Assay Principle

The Poly (ADP-ribose) ELISA kit is a sandwich ELISA for the quantitative measurement of PAR. The samples or PAR standards are first added to a monoclonal anti-PAR antibody coated microplate. After a brief incubation, an anti-PAR polyclonal detection antibody is added, followed by an HRP conjugated secondary antibody. After adding the substrate a colorimetric reaction takes place that can be measured with a colorimetric plate reader. The PAR content in unknown samples is determined by comparing this absorbance with the ones from the PAR polymer standard curve.

Related Products

1. MBS169272: NAD+/NADH Assay Kit (Colorimetric)
2. MBS169286: NAD+/NADH Assay Kit (Fluorometric)
3. MBS168191: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
4. MBS168496: OxiSelect™ DNA Double-Strand Break (DSB) Staining Kit
5. MBS168006: OxiSelect™ UV-induced DNA Damage ELISA Kit (CPD Quantitation)
6. MBS168678: OxiSelect™ UV-induced DNA Damage ELISA Kit (6-4PP Quantitation)
7. MBS168757: OxiSelect™ Comet Assay Kit (3-Well Slides)
8. MBS168155: OxiSelect™ 96-Well Comet Assay Kit

Kit Components

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate
2. Anti-Poly (ADP-Ribose) Coating Antibody (Part No. 51146C): One 20 µL vial
3. Anti-Poly (ADP-Ribose) Detection Antibody (Part No. 51142C): One 15 µL vial
4. Secondary Antibody, HRP Conjugate (1000X) (Part No. 51143C): One 15 µL vial
5. Assay Diluent (Part No. 310804): One 50 mL bottle
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle
8. Stop Solution (Part. No. 310808): One 12 mL bottle
9. 100X PARP Inhibitor (Part No. 51145C): One 3 mL amber bottle of 3-aminobenzamide (3-AB) at 100 mg/mL (735 mM) in DMSO

10. Poly (ADP-Ribose) Standard (Part No. 51144D): One 10 µL vial at 2.5 µM.

Materials Not Supplied

1. Samples such as cell or tissue lysate
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
6. RIPA buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1%sodium deoxycholate, 0.1% SDS, Protein Inhibitors or Cocktail)
7. Sodium dodecyl sulfate (SDS)
8. 1X PBS

Storage

Upon receipt, store the Poly (ADP-Ribose) Standard at -80ºC. Store the antibodies at -20ºC. It is recommended to make aliquots to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-Poly (ADP-Ribose) Detection Antibody and Secondary Antibody, HRP Conjugate (1000X): Immediately before use, dilute the Detection Antibody and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.
- Anti-Poly (ADP-Ribose) Antibody Coated Plate:

  Note: The Anti-Poly (ADP-Ribose) coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately. It is recommended that the user prepare the standards and samples during blocking of the Anti-Poly (ADP-Ribose) antibody coated plate.

  1. Immediately before use, dilute the Anti-Poly (ADP-Ribose) Coating Antibody 1:500 in 1X PBS. Example: Add 10 µL to 4.990 mL of 1X PBS. Vortex thoroughly.
  2. Add 100 µL of the mixture to each well to be tested and incubate overnight at 4ºC. Remove the Anti-Poly (ADP-Ribose) coating solution and blot the plate on paper towels to remove excess fluid.
  3. Add 200 µL of Assay Diluent to each well to be tested and block for 2 hr at room temperature. Remove the solution and wash the wells three times with 200 µL 1X PBS and blot the plate on paper towels to remove excess fluid. Use the plate immediately after the last PBS wash.

Preparation of Standard Curve

Prepare a dilution series of Poly (ADP-Ribose) standards in the concentration range of 5 nM to 0.019 nM by diluting the Poly (ADP-Ribose) Standard (2.5 µM) in Assay Diluent (Table 1).

Standard Tubes	Poly (ADP-Ribose) Standard (µL)	Assay Diluent (µL)	Poly (ADP-Ribose) (nM)
1	2	998	5
2	400 of Tube #1	400	2.5
3	400 of Tube #2	400	1.25
4	400 of Tube #3	400	0.625
5	400 of Tube #4	400	0.312
6	400 of Tube #5	400	0.156
7	400 of Tube #6	400	0.078
8	400 of Tube #7	400	0.039
9	400 of Tube #8	400	0.019
10	0	400	0

Table 1. Preparation of Poly (ADP-ribose) Standards

Preparation of Samples

- Cell Samples: Aspirate medium. Wash the cells once with PBS containing 1X PARP Inhibitor (1 mg/mL 3-AB). Add 1 ml of RIPA Buffer containing 1X PARP Inhibitor (1mg/mL 3-AB) to every 10 cm culture dish. Incubate on ice for 10-20 minutes. Scrape cells off the surface with a cell scraper. Transfer to a centrifuge tube and centrifuge at 10,000 g for 10 min at 4°C. Collect the supernatant, add SDS to a final concentration of 1% and boil (100°C) for 5 minutes. Snap-cool the tube on ice after boiling and then centrifuge at 10,000 g for 5 min. The supernatant can be assayed directly.
- Tissue Samples: Rapidly freeze tissues after collection (e.g., using liquid nitrogen). Weigh the frozen tissue and add 5-10 µL of RIPA Buffer containing 1X PARP Inhibitor (1mg/mL 3-AB) per mg of tissue. Sonicate or homogenize tissue sample (keeping it on ice) and centrifuge at 10,000 g for 10 minutes at 4°C. Collect the supernatant, add SDS to a final concentration of 1% and boil (100°C) for 5 minutes. Snap-cool the tube on ice after boiling and then centrifuge at 10,000 g for 5 min. The supernatant can be assayed directly.

  Note: 3-aminobenzamide (3-AB) is an inhibitor of PARP that will avoid artifactual synthesis of PAR during lysis of samples.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each Poly (ADP-Ribose) sample including unknown and standard should be assayed in triplicate.

2. Add 100 µL of unknown sample or Poly (ADP-Ribose) standard to the wells of the freshly prepared Poly (ADP-ribose) antibody coated plate. Incubate at room temperature for 1 hour on an orbital shaker.
3. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted anti-Poly (ADP-Ribose) Detection Antibody to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 3 above.
5. Add 100 µL of the diluted Secondary Antibody-HRP conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Warm Substrate Solution to room temperature during this incubation.
6. Wash microwell strips 3 times according to step 3 above. Proceed immediately to the next step.
7. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation. A nice gradient of blue color on the standard curve should be visible before adding the stop solution.
8. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
9. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Poly (ADP-Ribose) ELISA results. One should use the data below for reference only. These data should not be used to interpret actual results.

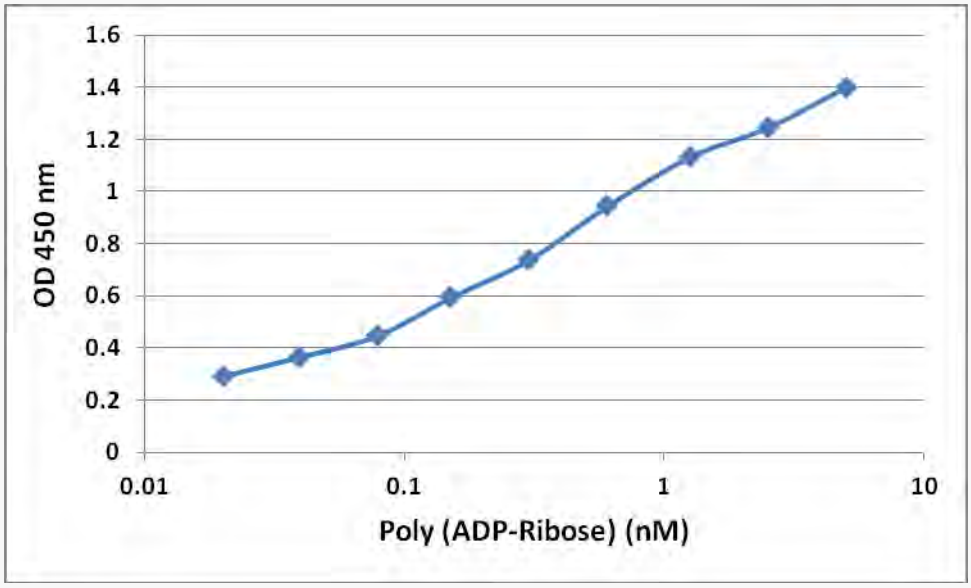

Figure 2: Poly (ADP-Ribose) ELISA Standard Curve.

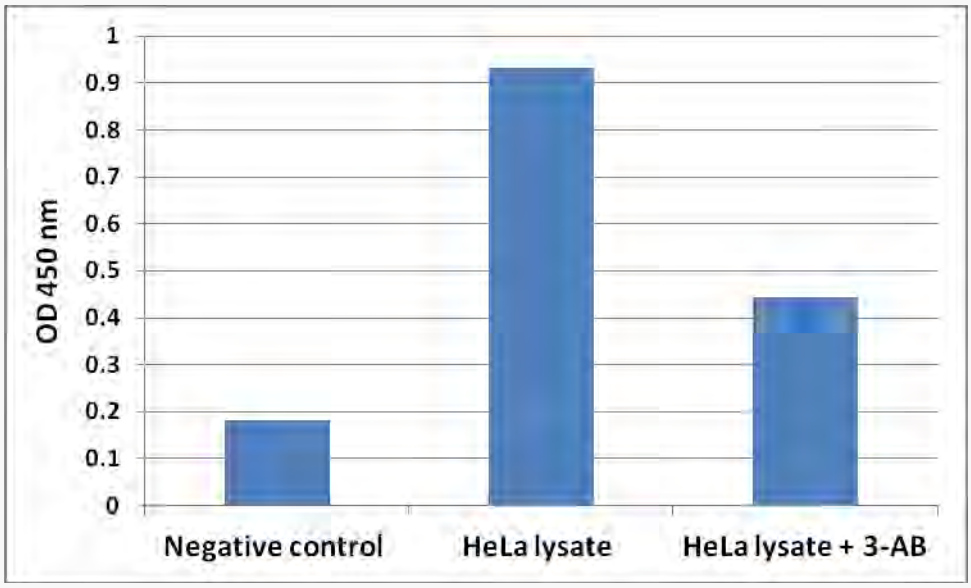

Figure 3: Poly (ADP-Ribose) levels in HeLa cells lysate. HeLa cells lysates were prepared in RIPA Buffer with or without PARP Inhibitor according to the sample preparation section. The negative control is the lysis buffer used to lyse the cells (no cells).

References

1. Miwa, M. and Masutani, M. (2017). PolyADP-ribosylation and cancer. *Cancer Sci*. **98(10)**:1528-35.
2. Lüscher, B. et al. (2018). ADP-Ribosylation, a Multifaceted Posttranslational Modification Involved in the Control of Cell Physiology in Health and Disease. *Chem Rev.* **118(3)**:1092-1136.
3. Martin-Hernandez, K. et al. (2017). Expanding functions of ADP-ribosylation in the maintenance of genome integrity. *Semin Cell Dev Biol.* **63**:92-101.

4. Ohmoto, A. and Yachida, S. (2017). Current status of poly(ADP-ribose) polymerase inhibitors and future directions. *Onco Targets Ther*. **26(10)**:5195-5208.

Recent Product Citations

1. Lucien, F. et al. (2022). Poly (ADP-Ribose) and α-synuclein extracellular vesicles in patients with Parkinson disease: A possible biomarker of disease severity. *PLoS One*. **17**(4):e0264446. doi: 10.1371/journal.pone.0264446.
2. Li, H. et al. (2021). Striatal oxidative damages and neuroinflammation correlate with progression and survival of Lewy body and Alzheimer diseases. *Neural Regen Res*. **17**(4):867-874. doi: 10.4103/1673-5374.322463.
3. Yu, Y. et al. (2021). Parp mutations protect from mitochondrial toxicity in Alzheimer's disease. *Cell Death Dis*. **12**(7):651. doi: 10.1038/s41419-021-03926-y.