Description

LysA™ Universal PARylation Assay Kit is a sandwich ELISA-based kit designed to measure and quantify the amount of total poly ADP-ribosylation present in cell extracts. The kit comes in a convenient 96-well format and contains all the reagents needed, including a PAR standard that allows to establish a standard curve for quantitative measurements. It contains enough antibodies, blocking buffer, and detection reagents to measure PARylation levels in cell extracts, in a 96 well plate format. It also includes cell lysates as controls for assay performance.

Note: The assay is adequate for Poly-ADP-ribosylation detection only. The assay is linear in the 100 pM to 20 nM PARylation range.

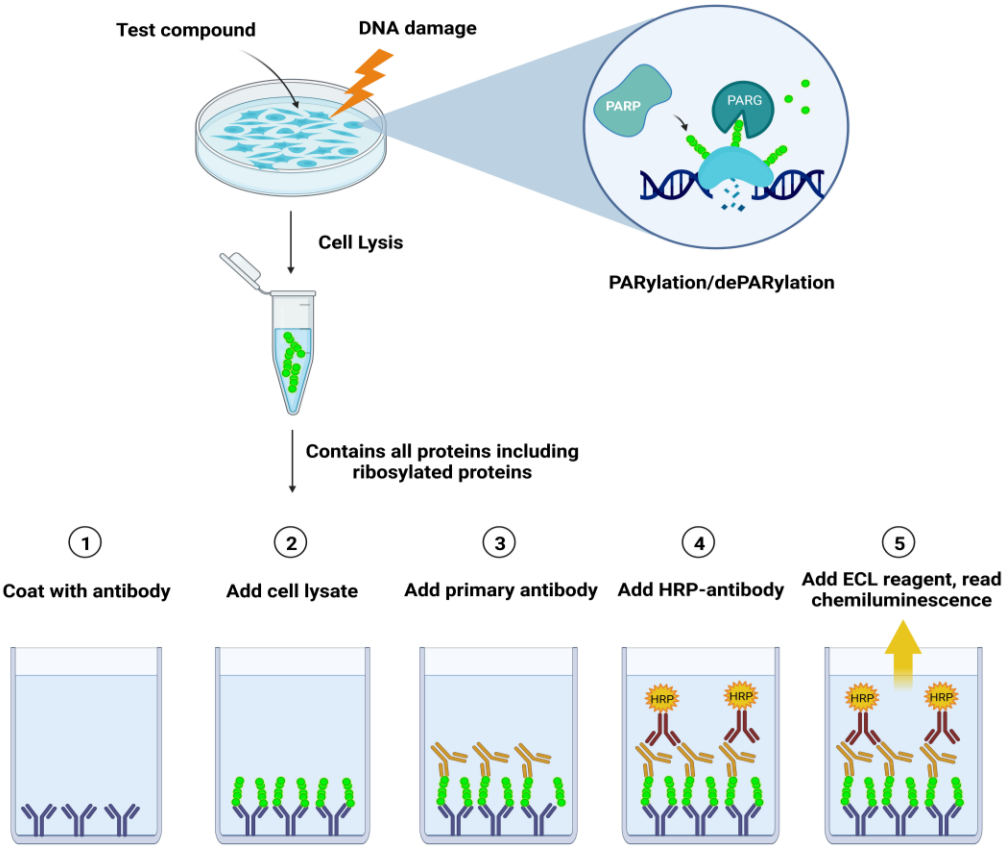

Figure 1: LysA™ Universal PARylation Assay workflow diagram.

An anti-PAR antibody is used to coat the 96-well plate. Lysates from cells are added to the coated wells, so PAR (PARylated proteins) present in the cell lysates are captured by the antibody. This is followed by an incubation with an anti-PAR primary antibody and a secondary HRP-labeled antibody. Addition of a chemiluminescent HRP substrate provides a luminescence signal, which directly correlates with the amount of PAR present in the cell extracts.

Background

PAR homeostasis is regulated by the family of PAR polymerases (PARPs) and PARG (Poly (ADP-ribose) glycohydrolase) in response to cellular stress conditions. ADP ribosylation, which is the addition of an ADP-ribose to a protein, is a reversible post-translational modification of proteins mostly involved in the DNA Damage Response (DDR) pathway. Poly-ADP-ribosylation (termed PARylation) is the addition of linear or branched chains of ADP-ribose. PARP and PARG activity are linked to cellular responses in inflammation, ischemia, stroke, and cancer. PARP inhibitors have been used in cancer treatment with success, leading to synthetic lethality when homologous recombination repair (HRR) is already defective. PARG is overexpressed in breast cancer and associated with tumor growth and survival. Decrease in PARG activity can potentiate the effect of current cancer therapies, such as chemotherapy and radiation, making PARG inhibition with selective inhibitors a promising approach in cancer and immunotherapy. Further studies of PARP and PARG will elucidate how the levels of PARylated proteins contribute to disease and can be modulated to provide therapeutic benefit.

Applications:

- Quantify total levels of PAR in cultured cells.
- Screen or determine the IC_{50} of PARP or PARG inhibitors in cultured cells.
- Validation of PARP or PARG inhibitors in a cellular model.

Supplied Materials

Catalog #	Name	Amount	Storage
	10 µM PAR Standard	7 µl	-80°C
	Anti-PAR Coating Antibody	10 µl	-80°C
	Anti-PAR Detection Antibody	10 µl	-80°C
82156	HEK293 High PAR Cell Lysate	300 µl	-80°C
82157	HEK293 Low PAR Cell Lysate	300 µl	-80°C
52131H	Secondary HRP-Labeled Antibody 2	10 µl	-80°C
79743	Blocking Buffer 3	2 x 25 ml	+4°C
79670	ELISA ECL Substrate A	6 ml	Room Temp
	ELISA ECL Substrate B	6 ml	Room Temp
79837	96-well module Maxisorp™ plate		Room Temp

Materials Required but Not Supplied

- Test Samples (e.g cell lysates)
- 1x PBS (Phosphate Buffer Saline)
- PBST Buffer (1x PBS with 0.05% Tween-20)
- Microplate reader capable of reading luminescence
- Adjustable micropipettor and sterile tips
- Orbital shaker

Materials Not Supplied But Recommended for Cell Lysate Preparation

Name	Ordering Information
Modified RIPA Lysis Buffer (Moderate Strong)	BPS Bioscience #82126
LysA™ Protease Inhibitor Cocktail Kit	BPS Bioscience #82199
ADP-Ribosylation Cycle Inhibitor Mix	BPS Bioscience #82130

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- This assay does not allow detection of mono-(ADP-ribose) (MAR) modifications on proteins.
- Avoid SDS or other strongly denaturing detergents at concentration > 0.1%.
- This assay is compatible with up to 2% v/v total detergent present and up to 1% DMSO.
- Avoid the use of sodium azide and reducing agents, such as dithiothreitol or β-mercaptoethanol at concentration >10 mM.

Cell Lysate Collection

- We suggest using 1-10 x 10^5 cells per condition in a cell lysate volume of 50 µl. The appropriate amount of cell extract may require adjustment, as PAR levels may vary with cell type, culture conditions, and treatments.
- Variation in sample collection, processing and storage may cause differences in sample values.
- Artifactual PAR synthesis or PAR removal during sample processing can occur. We strongly recommend the addition of a protease inhibitor cocktail, such as LysA™ Protease Inhibitor Cocktail Kit (BPS Bioscience #82199), to prevent protein degradation and an ADP-ribosylation cycle inhibitor mix (BPS Bioscience #82130) to the cell lysis buffer prior to homogenization/cell lysis.
- Modified RIPA Lysis Buffer (BPS Bioscience #82126) is compatible with this assay. Other lysis buffers (such as RIPA) may be used; however, optimization of the lysis condition and dilution factors may be required.
- **The sample collection protocol described here is a general guideline only.**

1. Wash cells with ice-cold PBS.

2. Add LysA™ Protease Inhibitor Cocktail Kit to the cell lysis buffer of choice, for example Modified RIPA Lysis Buffer (Moderate Strong).

3. Add ADP-Ribosylation Cycle Inhibitors Mix, or other ADP-ribosylation inhibitors of choice.

4. Transfer cells to ice.

5. Lyse cells in each well with 50 µl of complete cell lysis buffer.

6. Incubate on ice for at least 10 minutes.

7. Ensure all cells have been lysed by pipetting up and down or other methods.

8. It is recommended to assay immediately for total PAR. If necessary, store the cell lysates at -80°C before use.

ELISA Assay Protocol

- All conditions should be tested in triplicate, except standards (these can be run in duplicate).
- Coat the plate one day prior to analyzing your samples using the ELISA kit.
- It is recommended to generate a PAR Standard Curve, by performing a 2-3-fold serial dilution.
- This assay should include "Background Control" (condition with no cell lysate), "Positive Control Cell Lysate", "Negative Control Cell Lysate", "PAR Standard" (if applicable) and "Test Cell Lysate" conditions.
- "Test Cell Lysate" refers to all cellular conditions to be tested (those include untreated control cells and other appropriate experimental conditions of the cellular experiment).

Step 1: Plate coating

1. Dilute **Anti-PAR Coating Antibody** 500-fold with PBS (50 µl/well).

2. Add 50 µl of diluted Anti-PAR Coating Antibody to each well and incubate at 4°C overnight.

3. Wash each well three times with 200 µl PBST Buffer.

4. Tap the plate onto clean paper towel to remove the liquid.

5. Add 200 µl of Blocking Buffer 3 to every well.

6. Incubate at Room Temperature (RT) for at least 2 hours.

7. Wash each well three times with 200 µl PBST Buffer.

8. Tap the plate onto clean paper towel to remove the liquid.

9. The plate is now ready and should be used immediately.

Step 2: Preparation of PAR standard (if applicable)

- The assay is linear in the 100 pM to 20 nM PARylation range.
- Measured values for standard or samples that fall outside of this range are not accurate and require the sample to be diluted or concentrated (as needed).

1. Thaw the **PAR Standard** on ice. Briefly spin the tube to recover the full content of the tube.

2. Dilute the PAR Standard 500-fold in the cell lysis buffer used in sample preparation. This will correspond to the highest value on the standard curve.

3. Prepare a serial dilution of the diluted PAR Standard using the cell lysis buffer as diluent (50 µl/well).

Step 3: Assay

1. Add 50 µl of cell lysis buffer to wells labeled ""Background Control" (no cell lysate).

2. Add 50 µl of PAR Standard dilutions to the wells labeled "PAR Standard".

3. Add 50 µl of each test cell lysate to the wells labeled " Test Cell Lysate".

4. Add 50 µl of HEK293 High PAR Cell Lysate to the "Positive Control Cell Lysate" wells.

5. Add 50 µl of HEK293 Low PAR Cell Lysate samples to the "Negative Control Cell Lysate" wells.

6. Incubate the plate at RT with slow agitation for 1 hour.

7. Wash the plate three times with 200 µl PBST Buffer.

8. Block the wells by adding 200 µl of Blocking buffer 3 to every well and incubate at RT for at least 20 minutes.

   Note: If BPS Bioscience's Modified RIPA Lysis Buffer is used and strong readouts are expected from positive wells this step can be omitted.

9. Dilute **Anti-PAR Detection Antibody** 500-fold in Blocking Buffer 3.

10. Add 50 µl to each well.

11. Incubate at RT with gentle agitation for 45 minutes to 1 hour.

12. Wash the plate three times with 200 µl PBST Buffer.

13. Tap the plate onto clean paper towel to remove the liquid.

14. Dilute 1,000-fold the **Secondary HRP-Labeled Antibody 2** with Blocking Buffer 3 (50 µl/well).

15. Add 50 µl of diluted Secondary HRP-Labeled Antibody 2 to each well.

16. Incubate at RT with agitation for 30-45 minutes.

17. Wash the plate three times with 200 µl PBST Buffer.

18. Tap the plate onto clean paper towel to remove the liquid.

19. Mix 1 volume of **ELISA ECL Substrate A** with 1 volume of **ELISA ECL Substrate B** (100 µl/well of mixture is needed).

20. Add 100 µl per well.

21. Immediately read the plate in a luminometer microtiter-plate reader capable of reading chemiluminescence.

22. Generate a standard curve of luminescence versus PAR values (nM) using linear regression and y-intercepting at zero.

Example Results

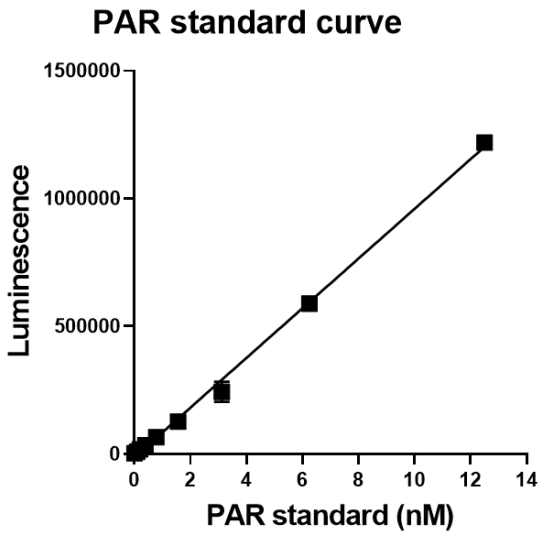

Figure 2: Example of PAR standard curve.
Various amounts of the PAR standard were run in duplicate. A linear response is seen between 100 pM to 20 nM.

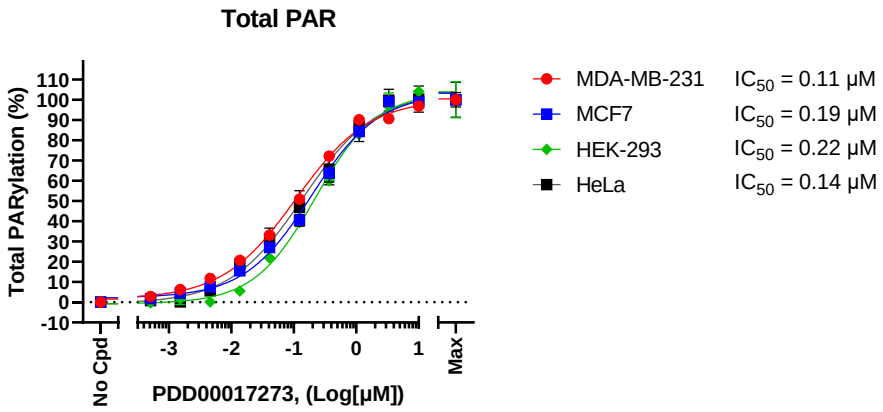

Figure 3: Effect of the PARG inhibitor PDD00017273 on cellular PARylation levels in various cell lines. HEK-293, MCF7, MDA-MB-231 and HeLa cells were plated in a 96 well plate at a cell density that allowed to reach approximately 70% confluency at the time of the experiment. Cells were treated with increasing concentrations of PDD00017273 (MedChemExpress #HY-108360), a PARG inhibitor, for 1 hour and 45 minutes, and an additional 15 minutes with 500 µM hydrogen peroxide. Cell extracts were collected in Modified RIPA Lysis Buffer containing ADP-Ribosylation Cycle Inhibitor Mix and analyzed. Luminescence was measured using a Bio-Tek microplate reader. Results are expressed as percent of total PARylation (in which the maximum PARylation level was set at 100%). For more information refer to our Cellular PARylation Tech Note.

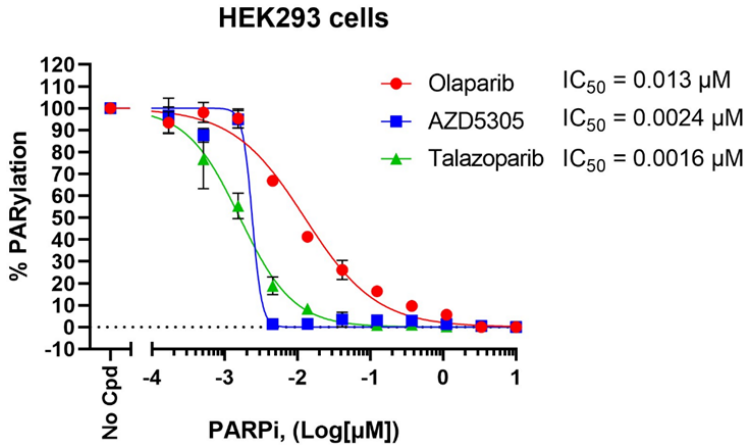

Figure 4: Effect of various PARP inhibitors on cellular PARylation levels in HEK293 cells.
HEK-293 cells were plated in a 96 well plate at a cell density that allowed to reach approximately 70% confluency at the time of the experiment. Cells were treated with increasing concentrations of Olaparib, AZD5305, and Talazoparib (BPS Bioscience #78318) for 1 hour 45 minutes and with 500 µM hydrogen peroxide for an additional 15 minutes. Cell extracts were collected in Modified RIPA Lysis Buffer containing ADP-Ribosylation Cycle Inhibitor Mix and analyzed. Luminescence was measured using a Bio-Tek microplate reader. Results are expressed as percent of PARylation levels (in which the no inhibitor control was set at 100%). For more information refer to our Cellular PARylation Tech Note.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

Marques M., *et al.*, 2019 *Oncogene* 38 (12): 2177-2191.
James D. I., *et al.*, 2016 *ACS Chem Biol* 11 (11): 3179-3190.
Drown B. S., *et al.*, 2018 *Cell Chem Bio* 25 (12): 1562-1570.

Related Products

Products	*Catalog #*	*Size*
PARG Fluorogenic Assay Kit	78858	96 reactions/384 reactions
PARPtrap™ Combo Assay Kit for PARP1 and PARP2	78317	384 reactions
PARPtrap™ Assay Kit for PARP1	80584	96 reactions/384 reactions
PARPtrap™ Assay Kit for PARP2	78296	96 reactions/384 reactions
ARH3 Fluorogenic Assay Kit	82158	96 reactions

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