Description

The PARP1 Colorimetric Assay Kit is designed to measure PARP1 (poly-(ADP-ribose) polymerase 1) activity for screening and profiling applications. The PARP1 assay kit comes in a convenient 96-well format, with enough purified PARP1 enzyme, reaction substrates and PARP assay buffer for 100 enzyme reactions.

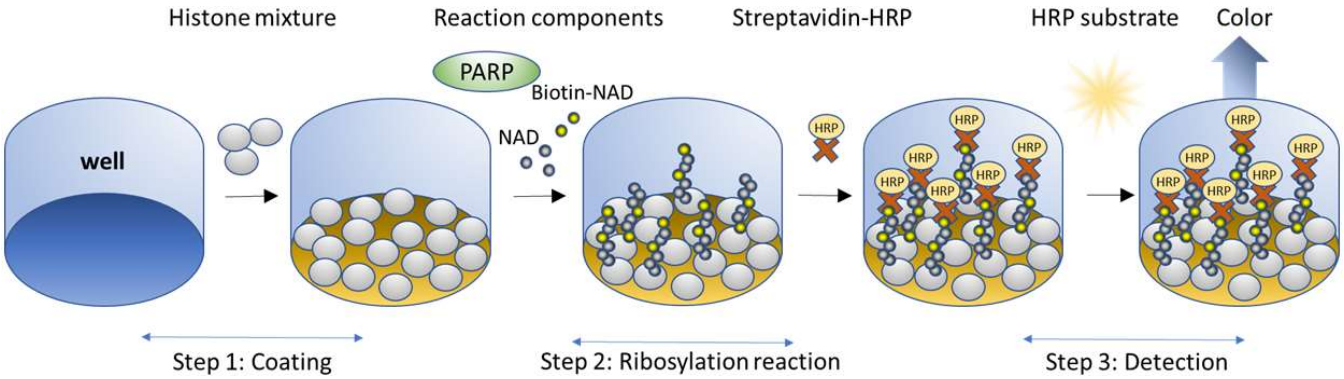

Figure 1. PARP1 Colorimetric Assay Kit schematic.

Histone proteins are coated on a 96-well plate. Next, a biotinylated NAD^+ mix (termed PARP Substrate Mixture) is incubated with the PARP1 enzyme and an activated DNA template in an optimized assay buffer. The plate is then treated with streptavidin-HRP followed by addition of the colorimetric HRP Substrate which generates a colored solution. The signal generated is proportional to PARP1 activity.

Background

PARP1, also known as poly-(ADP-ribose) polymerase 1 or NAD^+ ADP-ribosyltransferase 1, is part of the PARP family, and it is the most abundant member. 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. PARP1 participates in DNA repair by non-homologous end joining (NHEJ), homologous recombination (HR), microhomology-mediated end-joining (MMEJ) and nucleotide excision repair. Dysfunction of DDR pathways can lead to oncogenesis. Overexpression of PARP1 has been found in breast and colon cancer, neuroblastoma, and others. This overexpression can lead to increasing MMEJ, an error-prone DNA repair mechanism, and genome instability leading to cancer. In addition to being involved in DDR, PARP1 is also linked to inflammation and type I diabetes. PARP1 inhibitors have been used in cancer treatment with success. In addition to reducing MMEJ, the use of PARP1 inhibitors can lead to synthetic lethality when homologous recombination repair (HRR) mechanisms are already defective, as in the case of BRCA1 (breast cancer susceptibility protein type 1) and BRCA2 deficient cells. Further understanding of the molecular pathways involving PARP1, and this contribution to disease, will continue to pave the way for new therapies for PARP1-linked diseases.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
80501	PARP1, GST-Tag*	1 µg	-80°C
52029	5x Histone Mixture	1 ml	-80°C
78366	PARP Substrate Mixture 1	250 µl	-80°C
80602	10x PARP Assay Buffer	1 ml	-20°C
79743	Blocking Buffer 3	25 ml	+4°C
80605	Activated DNA	20 µl	-80°C
80611	Streptavidin-HRP	100 µl	+4°C
79651	Colorimetric HRP Substrate	10 ml	+4°C
	0.5 M DTT	200 µl	-20°C
79964	96-well transparent plate	1	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- 1x PBS (Phosphate Buffer Saline) Buffer
- PBST Buffer (1x PBS, containing 0.05% Tween-20)
- UV/Vis spectrophotometer microplate reader capable of reading absorbance at λ=450 nm*
- 2 M sulfuric acid (aqueous)
- Adjustable micropipettor and sterile tips
- Orbital Shaker

**Alternately, a spectrophotometer reading at 650 nm may be used, but the sensitivity of the assay will be greatly reduced.*

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

The PARP1 Colorimetric Assay Kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Blank", "Positive Control" and "Test inhibitor".
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Olaparib or AZD-5305 (#78318 or #27003) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1 x, 1 x and 10 x the IC_{50} value shown in the validation data below.

Step 1: Coat 96-well module

1. Dilute 5x Histone Mixture 5-fold with PBS to make 1x Histone Mixture (50 µl/well).
2. Add 50 µl of 1x Histone Mixture to each well.
3. Incubate at 4°C overnight.
4. Wash the plate three times using 200 µl of PBST Buffer per well.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Block the wells by adding 200 µl of Blocking Buffer 3 to every well.
7. Incubate at Room Temperature (RT) for at least 90 minutes.
8. Wash the plate three times with 200 µl of PBST Buffer per well.
9. Tap the plate onto clean paper towel to remove the liquid.

Step 2: Ribosylation reaction

1. Dilute Activated DNA 32-fold with PBS.
2. Dilute 0.5 M DTT 50-fold with distilled water. This makes 10 mM DTT.
3. Prepare a Master Mix (25 µl/well): N wells x (2.5 µl of 10x PARP Buffer + 2.5 µl of PARP Substrate Mixture 1 + 5 µl of diluted Activated DNA + 12.5 µl of distilled water + 2.5 µl of 10 mM DTT solution).
4. Add 25 µl of Master Mix to every well.
5. Prepare 1x PARP Buffer by adding 1 volume of 10x PARP Assay Buffer and 1 volume of 10 mM DTT solution to 8 volumes of distilled water.
6. Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

6.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in 1x PARP Assay Buffer, 10-fold more concentrated than the desired final concentrations.

For the positive and negative controls, use 1x PARP Assay Buffer (Diluent Solution).

OR

6.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x PARP Assay Buffer, to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x PARP Assay Buffer, to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x PARP Assay Buffer, (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

7. Add 5 µl of Test Inhibitor to each well labeled as “Test Inhibitor”.

8. Add 5 µl of Diluent Solution to the “Positive Control” and “Blank” wells.

9. Thaw PARP1 enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.

10. Dilute PARP1 enzyme to 0.33 ng/µl with 1x PARP Buffer (20 µl/well).

11. Initiate the reaction by adding 20 µl of diluted PARP1 enzyme to the wells designated “Positive Control” and "Test Inhibitor".

12. Add 20 µl of 1x PARP Buffer to the “Blank” wells.

13. Incubate at RT for 1 hour.

	Blank	**Positive Control**	**Test Inhibitor**
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x PARP Buffer	20 µl	-	-
Diluted PARP1 (0.33 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

14. Wash the plate three times with 200 µl of PBST Buffer per well.

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

Step 3: Detection

1. Dilute Streptavidin-HRP 50-fold with Blocking Buffer 3 (50 µl/well).
2. Add 50 µl of diluted Streptavidin-HRP to each well.
3. Incubate for 30 minutes at RT.
4. Wash the plate three times with 200 µl of PBST Buffer per well.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Add 100 µl of the colorimetric HRP substrate to each well.
7. Incubate the plate at the RT until blue color is developed in the "Positive Control" wells.

   Note: For PARP1, it normally takes 15~20 minutes to fully develop the color. However, the optimal incubation time may vary, and should be determined empirically by the user.

8. Add 100 µl of 2 M sulfuric acid to each well.
9. Read the absorbance at 450 nm using a UV/Vis spectrophotometer microplate reader.

   Note: The "Blank" absorbance value should be ~0.05 at 450 nm. Alternatively, the plate may be read at 650 nm without adding 2 M sulfuric acid, but the Signal-to-Background ratio will be decreased.

Example Results

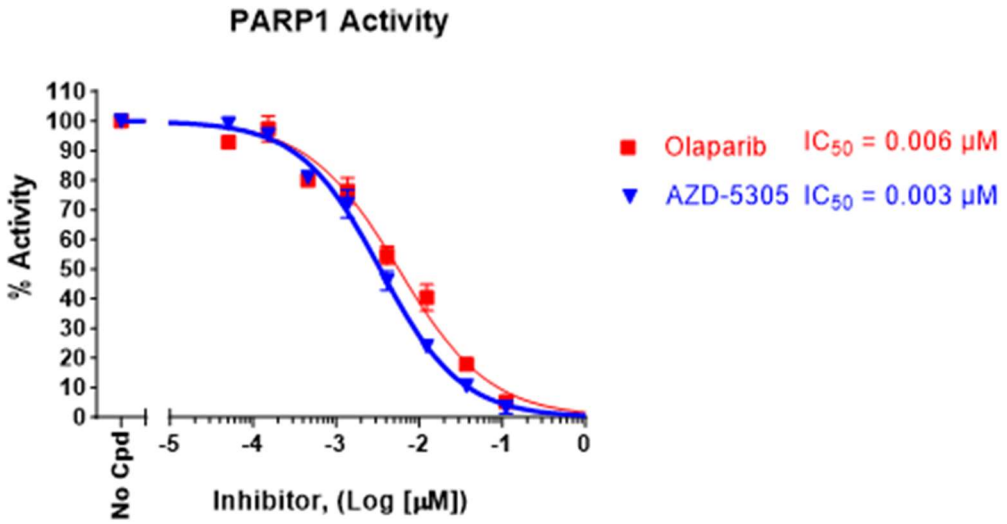

Figure 2: Inhibition of PARP1 activity by Olaparib and AZD-5305.
PARP1 was incubated with increasing concentrations of Olaparib and AZD-5305 (#78318). Absorbance was measured using a Tecan UV/Vis spectrophotometric microplate reader.

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.

Related Products

Products	*Catalog #*	*Size*
PARP2 Chemiluminescent Assay Kit	80552	96 reactions/384 reactions
PARP3 Chemiluminescent Assay Kit	80553	96 reactions/384 reactions
PARP6 Chemiluminescent Assay Kit	80556	96 reactions
PARP2, GST-Tag Recombinant	80502	10 µg
PARP3, GST-Tag Recombinant	80503	10 µg
PARP6, GST-Tag Recombinant	80506	10 µg

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