Description

The PARP2 Chemiluminescent Assay Kit is designed to measure the activity of PARP2 (poly-(ADP-ribose) polymerase 2) for screening and profiling applications. The PARP2 assay kit comes in a convenient 96-well format, with enough recombinant purified PARP2 enzyme (amino acids 2-583), reaction substrates and PARP assay buffer for 100 enzyme reactions.

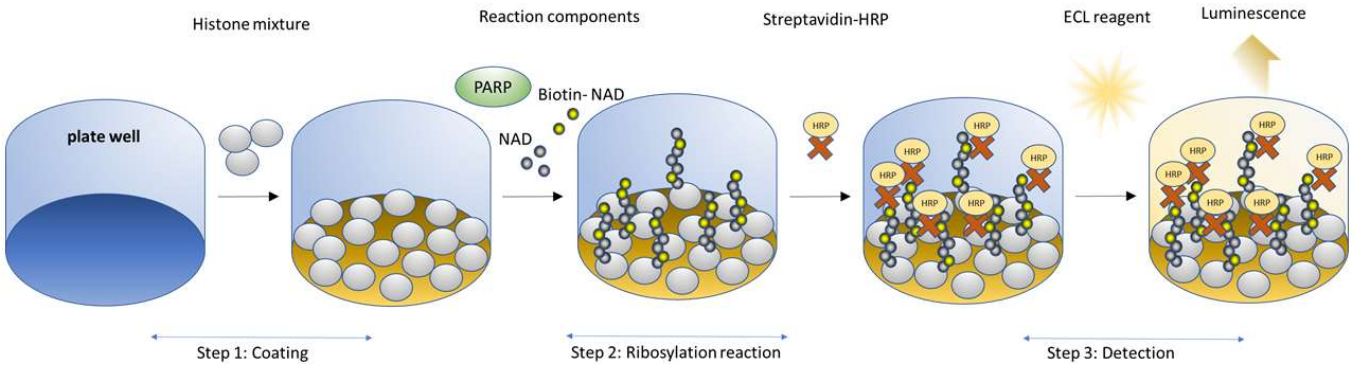

Figure 1. PARP2 Chemiluminescent Assay Kit schematic.

Histone proteins are coated on a 96-well plate. Next, a biotinylated NAD^+ mix (termed PARP Substrate Mixture) is incubated with the PARP2 enzyme and an activated DNA template in an optimized assay buffer. The plate is then treated with streptavidin-HRP followed by addition of the ELISA ECL substrate to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to the PARP2 activity.

Background

PARP2, also known as poly-(ADP-ribose) polymerase 2 or NAD^+ ADP-ribosyltransferase 2, is part of the PARP family. 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. PARP2 participates in DNA repair (only 10% of the PARP activity is due to PARP2), but also in oxidative stress and mitochondrial fragmentation. Dysfunction of the DDR and oxidative stress pathways can lead to oncogenesis. Genetic ablation of PARP2 has indicated roles of PARP2 in adipogenesis, spermatogenesis and thymocyte survival. It is also a co-factor of nuclear receptors like ER (estrogen receptor) and PPAR (peroxisome proliferator-activated receptors). PARP2 is overexpressed in prostate cancer (PCa) and may contribute to the disease through the FOXA1 (forkhead box protein A1)/AR pathway. PARP inhibitors have been used in cancer treatment with success, with the clinically approved inhibitors targeting both PARP1 and PARP2. Further understanding of the molecular pathways involving PARP2, and its contribution to disease, will continue to pave the way for new therapies for PARP2-linked diseases.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
80502	PARP2, GST-Tag*	1 µg	-80°C
52029	5x Histone Mixture	1 ml	-80°C
78366	PARP Substrate Mixture 1	2 x 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
	0.5 M DTT	200 µl	-20°C
80611	Streptavidin-HRP	100 µl	+4°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79837	96-well module 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)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

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 PARP2 Chemiluminescent Assay Kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control”, and “Test Inhibitor” conditions.
- 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 (#78318 or #27003) or AZD-5305 (#78318) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X 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. This makes 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 with 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 a 10 mM DTT solution.
3. Prepare a Master Mix (25 µl/well): N wells x (2.5 µl of 10x PARP Assay Buffer + 5 µl of PARP Substrate Mixture 1 + 5 µl of diluted Activated DNA + 10 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 Assay 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 soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration in 1x PARP Assay Buffer.

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

OR

6.2 If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute it 10-fold with 1x PARP Assay Buffer (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than 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 **PARP2** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.

10. Dilute PARP2 enzyme to **0.23 ng/µl** with 1x PARP Assay Buffer (20 µl/well).

11. Initiate the reaction by adding 20 µl of diluted PARP2 enzyme to the wells designated "Positive Control" and "Test Inhibitor."

12. Add 20 µl of 1x PARP Assay 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 Assay Buffer	20 µl	-	-
Diluted PARP2 (0.23 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 and tap the plate onto clean paper towel.

Step 3: Detection

1. Dilute Streptavidin-HRP 50-fold in Blocking Buffer 3 (50 µl/well).

2. Add 50 µl of diluted Streptavidin-HRP to every well.

3. Incubate for 30 minutes at RT.

4. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.

5. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).

6. Add 100 µl of mix to every well.

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

8. The "Blank" value should be subtracted from all other values.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example Results

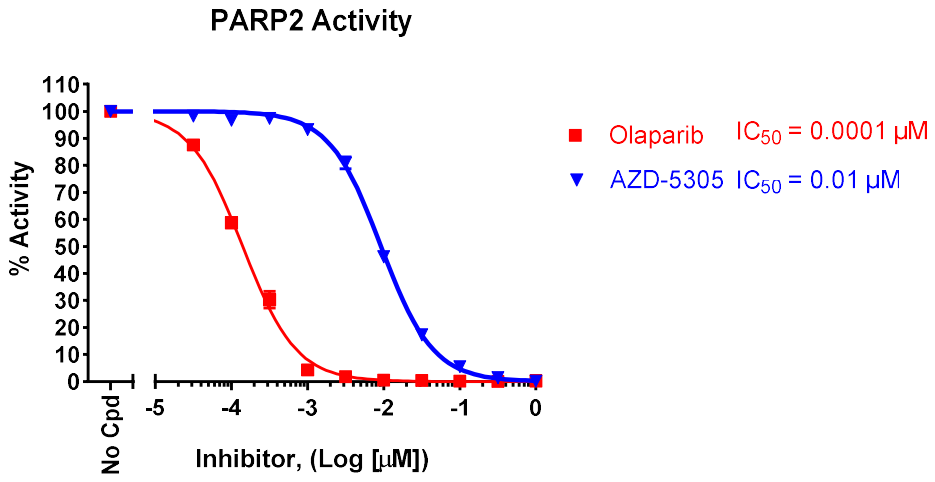

Figure 2: Inhibition of PARP2 activity by Olaparib and AZD-5305.
PARP2 was incubated with increasing concentrations of Olaparib (#78318 or #27003) and AZD-5305 (#78318). Luminescence was measured using a Bio-Tek microplate reader.

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

References

Gui B., *et al.*, 2019 *Proc Natl Acad Sci USA* 116 (29): 14573-14582.

Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
PARP2 Homogeneous Assay Kit	78572	384 reactions
PARP2 Colorimetric Assay Kit	80581	96 reactions
PARPtrap™ Assay Kit for PARP2	78296	96 reactions/384 reactions
PARP1 Chemiluminescent Assay Kit	80551	96 reactions
PARP3 Chemiluminescent Assay Kit	80553	96 reactions/384 reactions
TNKS2 (PARP5B) Chemiluminescent Assay Kit	78406	96 reactions

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