Description

The PARP10 Chemiluminescent Assay Kit is designed to measure the activity of PARP10 (poly-(ADP-ribose) polymerase 10) for screening and profiling applications. The PARP10 assay kit comes in a convenient 96-well format, with enough recombinant purified PARP10 enzyme, reaction substrates, and PARP assay buffer for 100 enzyme reactions.

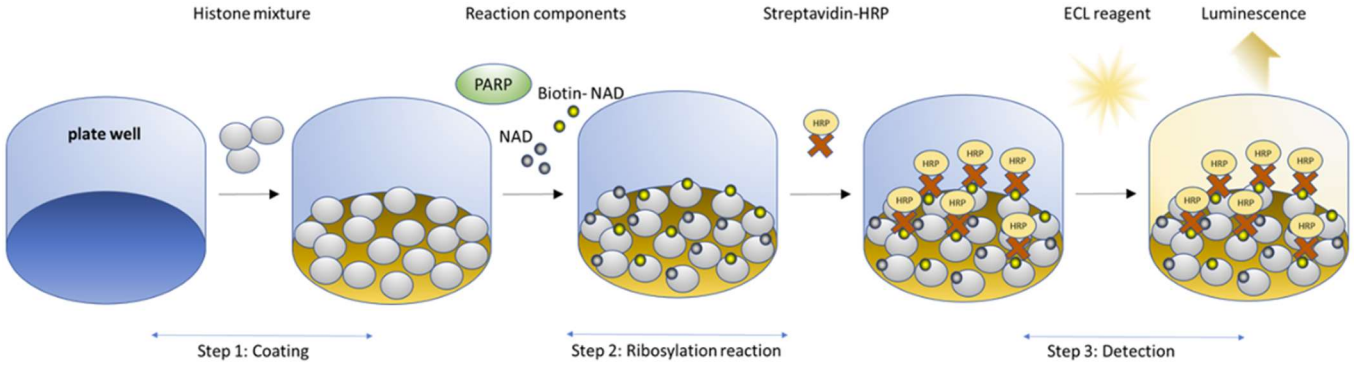

Figure 1. PARP10 Chemiluminescent Assay Kit schematic.

Histone proteins are coated on a 96-well plate. Next, a biotinylated NAD^+ mix (termed PARP Substrate Mixture) is incubated with the PARP10 enzyme 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 PARP10 activity.

Background

PARP10, also known as poly-(ADP-ribose) polymerase 10, NAD^+ ADP-ribosyltransferase 10 and ARTD10, 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. Mono-ADP-ribosylation (termed MARylation) is the addition of a unit of ADP-ribose. PARP10 is found both in the cytosol and the nucleus, having c-myc, histones, PCNA (proliferating cell nuclear antigen), GSK3β (gyclogen synthase kinase-3 beta) and other proteins as ligands. It is involved in metabolism, with lower levels of PARP10 linking to lower fatty acid oxidation, DNA damage and repair in response to hydroxyurea and UV light and maintenance of genome stability. PARP10 has also been linked to cancer, acting as a tumor suppressor by negatively regulating Aurora A and thus negatively regulating G2/M transition during cell cycle. Inhibition of PARP10 presents itself as a potential new tool in cancer therapy.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
80510	PARP10*	2 µg	-80°C
52029	5x Histone Mixture	1 ml	-80°C
78371	PARP Substrate Mixture 2	2 x 250 µl	-80°C
80602	10x PARP Assay Buffer	1 ml	-20°C
79743	Blocking Buffer 3	25 ml	+4°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 PARP10 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) 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 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 0.5 M DTT 50-fold with distilled water. This makes a 10 mM DTT solution.
2. Prepare a Master Mix (25 µl/well): N wells x (2.5 µl of 10x PARP Assay Buffer + 5 µl of PARP Substrate Mixture 2 + 15 µl of distilled water + 2.5 µl of 10 mM DTT solution).
3. Add 25 µl of Master Mix to every well.
4. 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.
5. 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.

   5.1 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

5.2 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 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%.

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

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

8. Thaw **PARP10** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.

9. Dilute PARP10 enzyme to **1 ng/µl** with 1x PARP Assay Buffer (20 µl/well).

10. Initiate the reaction by adding 20 µl of diluted PARP10 enzyme to the wells designated “Positive Control” and "Test Inhibitor."

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

12. 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 PARP10 (1 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

13. 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 each 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 per 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

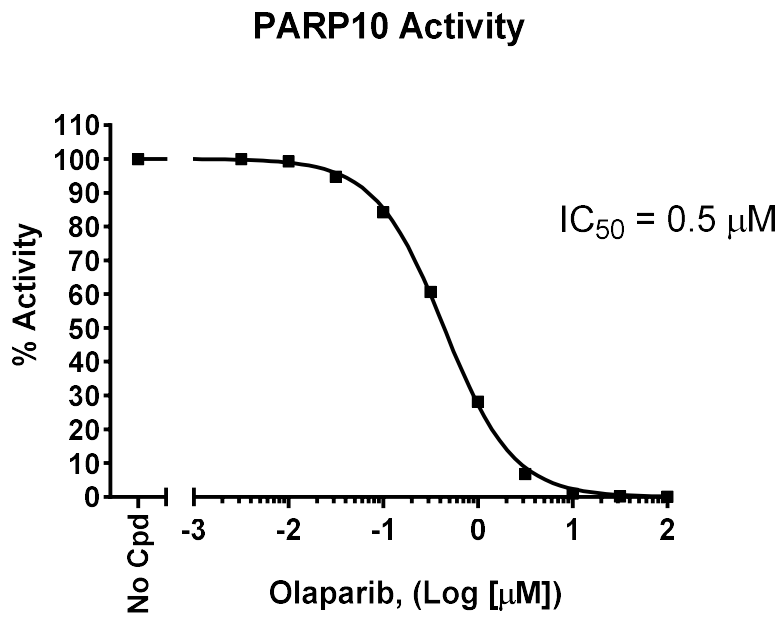

Figure 2: Inhibition of PARP10 activity by Olaparib.
PARP10 was incubated with increasing concentrations of Olaparib (#78318 or #27003). Luminescence was measured using a Bio-Tek microplate reader.

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

References

Marton J., *et al.*, 2018 *PLos One* 13(11): e0187789.
Zhao Y., *et al.*, 2018 *Oncogene* 37(22):2921-2935.
Dhoonmoon A. and Nicolae C., *NAR Cancer* 5(1):zcad009.

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*
PARP3 Homogeneous Assay Kit	78491	384 reactions
PARP1 Chemiluminescent Assay Kit	80551	96 reactions
PARP2 Chemiluminescent Assay Kit	80552	96 reactions/384 reactions
TNKS1 (PARP5A) Chemiluminescent Assay Kit	78405	96 reactions/384 reactions
TNKS2 (PARP5B) Chemiluminescent Assay Kit	78406	96 reactions

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