Description

The PARP3 Chemiluminescent Assay Kit is designed to measure PARP3 activity for screening and profiling applications. The PARP3 assay kit 96-well format contains enough purified PARP3 enzyme, histone mixture, activated DNA template, PARP assay buffer and necessary reagents for 100 enzyme reactions.

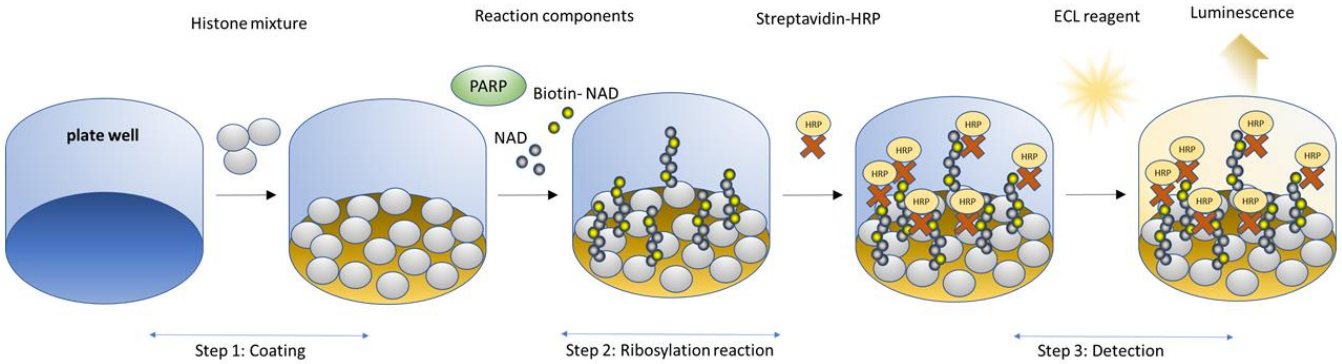

Figure 1. PARP3 Chemiluminescent Assay Kit schematic.

The key to the PARP3 Chemiluminescent Assay Kit is the biotinylated NAD^+ and involves 3 reactions. First, histone proteins are coated on a plate. Next, a biotinylated NAD^+ mix (termed PARP Substrate Mixture) is incubated with the enzyme and an activated DNA template. Finally, streptavidin-HRP is added, followed by ELISA ECL substrate to produce a chemiluminescence signal that can be measured using a chemiluminescence plate reader.

**NOTE: As of April 2022, this protocol has been re-optimized for performance. Previous versions of this kit are available upon request.*

Background

The (Poly-ADP-ribose) polymerase (PARP) family of proteins are involved in DNA repair, genomic stability, and programmed cell death. They catalyze the NAD-dependent addition of poly(ADP-ribose) to histones. PARP3 is critical in mitotic progression by stabilizing the mitotic spindle via regulation of NuMA and Tankyrase 1. PARP3 overexpression is found in patients with breast cancer and is associated with poor prognosis. PARP inhibition can be highly advantageous in tumors that involve BRCA1/2 (breast cancer type 1/2 susceptibility protein) mutations, as it leads to accumulation of DNA single strand breaks (SSBs) that are not repaired and cell death. PARP inhibitors work through “synthetic lethality”, where cell death is due to impaired function of two or more genes, but a defect in a single gene is not lethal. PARP3 inhibitors are thus therapeutical relevant in the treatment of metastatic breast cancer.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
80503	PARP3, GST-Tag*	2 x 1.25 µg	-80°C
52029	5x Histone Mixture	1 ml	-80°C
78366	10x PARP Substrate Mixture 1	4 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
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
78188	96-well plate		Room Temp

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

Materials Required but Not Supplied

- DTT (20 mM in water, prepared fresh)
- 1x PBS (phosphate buffer saline)
- PBST buffer (1x PBS, containing 0.05% Tween-20)
- Luminometer or plate 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. ***Avoid multiple freeze/thaw cycles!***

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 PARP3 Chemiluminescent Assay Kit is compatible with up to 1% final DMSO concentration. We recommend preparing the inhibitor in no higher than 10% DMSO solution in buffer and using 5 µl per well.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include a “Blank” and a “Positive control”.

Step 1: Coat the 384-well plate with histone solution

1) Dilute **5x Histone Mixture** 5-fold with PBS to make **1x Histone Mixture**.

2) Add 50 µl of **1x Histone Mixture** to each well and incubate at 4°C overnight.

3) Wash the plate three times using 200 µl of PBST buffer (1x PBS containing 0.05% Tween 20) per well.

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

5) Add 200 µl of **Blocking Buffer 3** to every well and incubate at Room Temperature for at least 90 minutes.

6) Wash the plate three times with 200 µl/well of PBST buffer.

7) Tap the plate onto clean paper towel to remove the liquid.

Step 2: Ribosylation reaction

1) Prepare a fresh solution of 20 mM DTT in distilled water.

2) Dilute **Activated DNA** 32-fold with PBS.

3) Prepare a Master Mix (25 µl/well): N wells x (2.5 µl of **10x PARP Buffer** + 10 µl of **10x PARP Substrate Mixture 1** + 5 µl of diluted Activated DNA + 5 µl of distilled water + 2.5 µl of 20 mM fresh DTT).

   Note: The concentration of DTT in the Master Mix will be 2 mM.

4) Add 25 µl of Master Mix to every well.

5) Prepare **1x PARP Buffer**: add one volume of 10x PARP Assay Buffer to one volume of 20 mM DTT and 8 volumes of distilled water.

   Note: The concentration of DTT in the 1x PARP Buffer will be 2 mM.

6) Prepare 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, dilute in 1x PARP Buffer at concentrations 10-fold higher than the final desired concentrations. The 1x PARP Buffer is the Diluent Solution.

   OR

   6.2. If the Test Inhibitor is soluble in DMSO, dissolve in 100% DMSO at a concentration 100-fold higher than the highest desired concentration. Then make a 10-fold dilution in 1x PARP Buffer. The compound concentration is 10-fold higher than the final desired concentration.

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

For positive and negative controls, prepare 10% DMSO in water (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 **PARP3** enzyme on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube.

10) Calculate the amount of PARP3 required for the assay (20 µl/well) and dilute enzyme to **1.1 ng/µl** with 1x PARP Buffer. Aliquot the remaining undiluted PARP3 enzyme in single use aliquots and store at -80°C. Do not re-use these aliquots more than once.

Note: PARP3 enzyme is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. ***Do not re-use the diluted enzyme.***

11) Initiate the reaction by adding 20 µl of diluted PARP3 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 Room Temperature 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 PARP3 (1.1 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 and tap the plate onto clean paper towel.

Step 3: Detection

1) Dilute **Streptavidin-HRP** 1:50 in Blocking Buffer 3.

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

3) Incubate for 30 minutes at Room Temperature.

4) Wash three times with 200 µl of PBST buffer and tap the plate onto clean paper towel.

5) Just before use, mix on ice 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B.

6) Add 100 µl to each 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

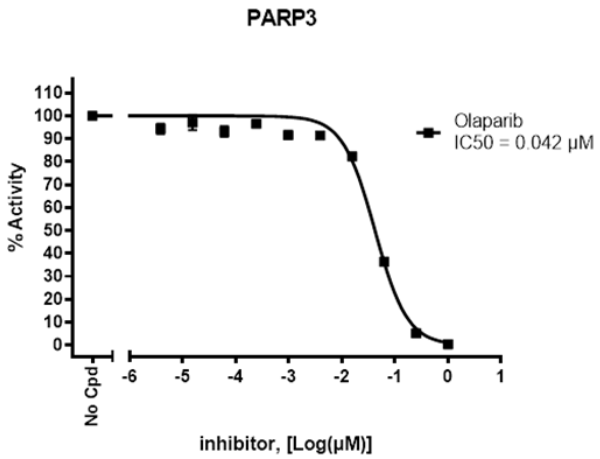

Figure 2: PARP3 activity in the presence of increasing concentrations of the inhibitor Olaparib. The inhibitory effect of Olaparib (LC Labs #O-9021) on PARP3 was measured using increasing amounts of inhibitor. Luminescence was measured using a Bio-Tek microplate reader. Results are expressed as percent of control activity (measured in the absence of inhibitor and set at 100%).

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 further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
PARP1 Chemiluminescent Assay Kit	80551	96 reactions
PARP3 Chemiluminescent Assay Kit	80553	96 reactions
TNKS1 (PARP5A) Chemiluminescent Assay Kit	78405	96 reactions
TNKS2 (PARP5B) Chemiluminescent Assay Kit	78406	96 reactions
PARP1, GST-Tag	80501	20 µg
PARP2, GST-Tag	80502	10 µg
PARP3, GST-Tag	80503	10 µg
Tankyrase 1 (PARP5A), GST-Tag	80504	10 µg
Tankyrase 2 (PARP5B) [849-1166], GST-Tag	80515	10 µg