Description

The PARP1 Chemiluminescent Assay Kit is designed to measure PARP1 activity for screening and profiling applications. PARP1 is known to catalyze the NAD-dependent addition of poly(ADP-ribose) to histones. The PARP1 assay kit comes in a convenient 384-well format, with purified PARP1 enzyme, histone mixture, activated DNA template, and PARP assay buffer for 400 enzyme reactions. The key to the PARP1 Chemiluminescent Assay Kit is the biotinylated NAD+. With this kit, only three simple steps are required for PARP1 reactions. First, histone proteins are coated on a 384-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. Finally, the plate is treated with streptavidin-HRP followed by addition of the ELISA ECL substrate to produce chemiluminescence that can be measured using a chemiluminescence reader.

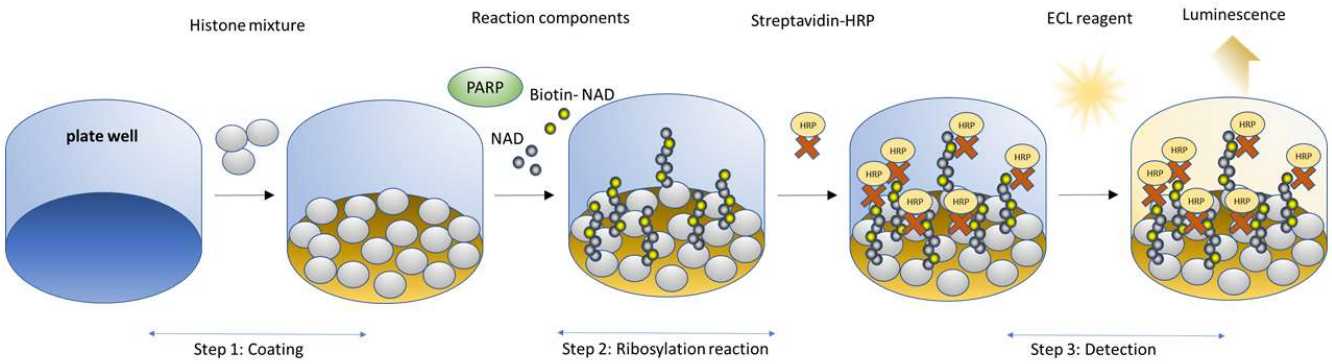

Figure 1. *PARP1 Chemiluminescent Assay Kit schematic*

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

Applications

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

BPS Bioscience
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Supplied Materials

Catalog #	Name	Amount	Storage	
80501	PARP1*	2 x 1 µg	-80°C	***Avoid multiple freeze/ thaw cycles***
52029	5x histone mixture	2 x 1 ml	-80°C	
78366	PARP Substrate Mixture 1	2 x 250 µl	-80°C	
80602	10x PARP assay buffer	2 x 1 ml	-20°C	
79743	Blocking buffer 3	2 x 25 ml	+4°C	
80605	Activated DNA	2 x 20 µl	-80°C	
80611	Streptavidin-HRP	2 x 100 µl	+4°C	
79670	ELISA ECL Substrate A (translucent bottle)	2 x 6 ml	Room Temp	
	ELISA ECL Substrate B (brown bottle)	2 x 6 ml	Room Temp	
78188	384-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 (10 mM in water, prepared fresh)
- 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. ***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 PARP1 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 2.5 µl per well.

Assay Protocol

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

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

1) Dilute 5x histone mixture 1:5 with PBS to make 1x histone mixture

2) Add 25 µl of histone mixture to each well and incubate at 4°C overnight

3) Wash the plate three times using 100 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) Block the wells by adding 100 µl of Blocking buffer 3 to every well. Incubate at room temperature for at least 90 minutes.

6) Wash the plate three times with 100 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 10 mM DTT in water.

2) Dilute Activated DNA 1:32 with PBS.

3) Prepare the Master Mix (12.5 µl/well): N wells x (1.25 µl of 10x PARP buffer + 1.25 µl of PARP Substrate Mixture 1 + 2.5 µl of diluted Activated DNA + 6.25 µl of water + 1.25 µl of 10 mM fresh DTT).

   Note: The concentration of DTT in the Master Mix will be 1 mM.

4) Add 12.5 µl of Master Mix to every well.

5) Prepare 1x PARP buffer with DTT. Dilute 10x PARP assay buffer to 1x PARP assay buffer containing DTT by adding 1 volume of 10x PARP assay buffer + 1 volume of 10 mM DTT + 8 volumes of water.

   Note: The concentration of DTT in the 1x PARP assay buffer will be 1 mM.

6) Add 2.5 µl of Test Inhibitor to each well labeled as “Test Inhibitor.”

   For the “Positive Control" and “Blank,” add 2.5 µl of the same diluent solution used to dilute the inhibitor, but without inhibitor (Diluent Solution).

   Note: The PARP1 Chemiluminescent Assay Kit is compatible with up to 1% final DMSO concentration. If the inhibitor is dissolved in DMSO, we recommend preparing the inhibitor in 10% DMSO aqueous solution and

using 2.5 µl per PARP1 reaction.

For example, if the compound is dissolved in DMSO, make a 100-fold higher concentration of the compound in DMSO than the highest concentration you want to test in the assay. Then dilute 10-fold in 1x PARP buffer (containing DTT). At this step the compound concentration is 10-fold higher than the desired final concentration. If you want to run an IC_{50} or test lower concentrations of the compound, prepare serial dilutions using 1x PARP buffer containing 10% DMSO, so the final concentration of DMSO will remain at 1% final.

If the compound is soluble in water, use the 1x PARP assay buffer containing DTT to prepare the test inhibitor.

7) Thaw PARP1 enzyme on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube. Calculate the amount of PARP1 required for the assay and dilute enzyme to **0.33 ng/µl** with 1x PARP buffer with DTT. The final concentration of PARP1 will be 1 nM. Aliquot the remaining undiluted PARP1 enzyme and store at -80°C. Do not re-use these aliquots more than once.

Note: PARP1 enzyme is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. ***Do not re-use the diluted enzyme.***

8) Initiate the reaction by adding 10 µl of diluted PARP1 enzyme to the wells designated “Positive Control” and "Test Inhibitor."

To the wells designated as "Blank," add 10 µl of 1x PARP buffer with DTT.

Incubate at room temperature for 1 hour.

	Blank	Positive Control	Test Inhibitor
Master Mix	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-
1x PARP buffer with DTT	10 µl	-	-
PARP1 (0.33 ng/µl)	-	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**

9) Wash the plate three times with 100 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 25 µl of diluted Streptavidin-HRP to each well. Incubate for 30 minutes at room temperature.

3) Wash three times with 100 µl of PBST buffer and tap the plate onto clean paper towel.

4) Just before use, mix on ice 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B. Add 50 µl per well.

5) Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence. The "Blank" value is 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

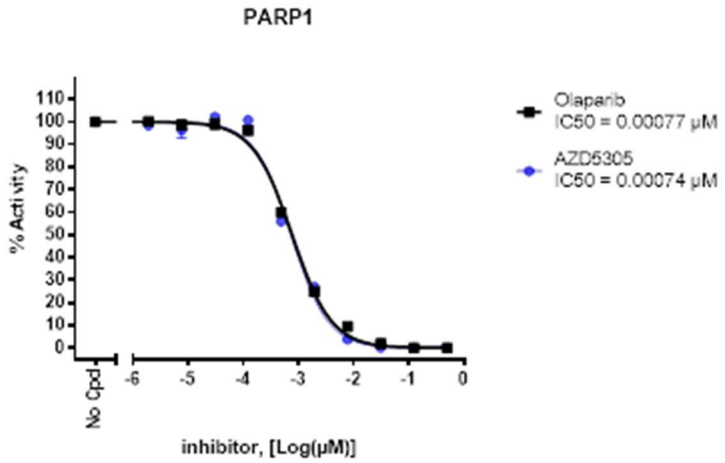

Figure 1: *PARP1 activity in the presence of increasing concentrations of Olaparib.* The effect of Olaparib (LC Labs, #O-9021) or AZD5305 (MedChemExpress, #HY-132167) was measured using the PARP1 Chemiluminescent Assay Kit (BPS Bioscience #80569) following the kit protocol. Luminescence was measured using a Bio-Tek microplate reader.

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.

Related Products

Products	*Catalog #*	*Size*
PARP2 Chemiluminescent Assay Kit	80552	96 rxns.
PARP3 Chemiluminescent Assay Kit	80553	96 rxns.
PARP6 Chemiluminescent Assay Kit	80556	96 rxns.
PARP7 Chemiluminescent Assay Kit	79729	96 rxns.
PARP10 Chemiluminescent Assay Kit	80560	96 rxns.
PARP11 Chemiluminescent Assay Kit	80561	96 rxns.
PARP14 Chemiluminescent Assay Kit	80568	96 rxns.
PARP15 Chemiluminescent Assay Kit	80567	96 rxns.
TNKS1 (PARP5A) Chemiluminescent Assay Kit	78405	96 rxns.
TNKS2 (PARP5B) Chemiluminescent Assay Kit	78406	96 rxns.
PARP1, GST-Tag	80501	20 µg
PARP2, GST-Tag	80502	10 µg
PARP3, GST-Tag	80503	10 µg
PARP6, GST-Tag	80506	10 µg
PARP7, FLAG-Tag	80527	10 µg
PARP10, FLAG-Strep-Tag	80522	10 µg
PARP11, GST-Tag, His-Tag	80511	10 µg
PARP12, His-GST-Tag	80513	10 µg
PARP14, His-GST-Tag	80514	10 µg
PARP15, GST-Tag	80517	10 µg
Tankyrase 1 (PARP5A), GST-Tag	80504	10 µg
Tankyrase 2 (PARP5B) [849-1166], GST-Tag	80515	10 µg