

**Description**

The Chemi-Verse™ PARP7 Assay Kit is designed to measure the activity of PARP7 (poly-(ADP-ribose) polymerase 7) for screening and profiling applications. The PARP7 assay kit comes in a convenient 96-well format, with enough recombinant purified PARP7 enzyme (amino acids 400-657), histone mixture, NAD<sup>+</sup>, a ribose-binding agent and PARP assay buffer for 100 enzyme reactions.

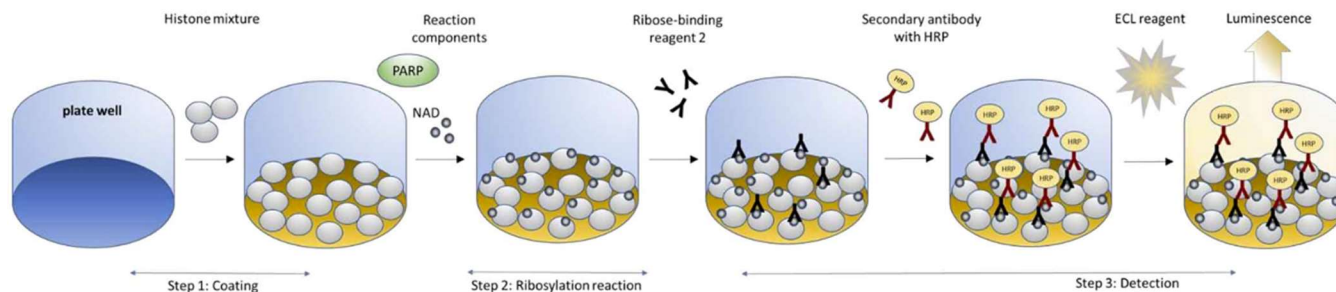


Figure 1. Chemi-Verse™ PARP7 Assay Kit schematic.

Histone proteins are coated on a 96-well plate. Next, a biotinylated NAD<sup>+</sup> substrate is incubated with the PARP7 enzyme in an optimized assay buffer. The plate is then treated with an ADP-ribose specific binding agent and a secondary HRP-conjugated antibody. ELISA ECL substrate is added to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to PARP7 activity.

**Background**

PARP7, also known as poly-(ADP-ribose) polymerase 7 or NAD<sup>+</sup> ADP-ribosyltransferase 7, 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. PARP7 is a negative regulator of nucleic acid sensing in cancer cells. Tumor cells contain cytosolic DNA as a reflection of defects in DNA repair and must repress responses coming from the adaptative immune system. PARP7 is an AHR (aryl hydrocarbon receptor) regulated gene that inhibits STING (stimulator of interferon genes) and IFN-I (interferon type I). RBN-2397 is a pyridazinone-based PARP7 inhibitor that results in STING-dependent and IFN-I activation and leads to cancer cell death *in vitro* and it is currently under clinical trial. The development of PARP7 inhibitors is thus a promising area in cancer research.

**Applications**

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

**Supplied Materials**

Catalog #	Name	Amount	Storage
80527	PARP7, FLAG-Tag*	2 µg	-80°C
52029	5x Histone Mixture	1 ml	-80°C
	50 µM NAD <sup>+</sup>	250 µl	-80°C
	PP-16-2 Assay Buffer	1 ml	-20°C
79743	Blocking Buffer 3	2 x 25 ml	+4°C
	0.5 M DTT	200 µl	-20°C
	Ribose-Binding Reagent 2-D	10 µl	-80°C
52131H	Secondary HRP-Antibody 2	10 µl	-80°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 Chemi-Verse™ PARP7 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 \(bpsbioscience.com\)](https://www.bpsbioscience.com).
- We recommend using RBN-2397 as internal control (#78318). 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<sub>50</sub> 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 every 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 PP-16-2 Assay Buffer + 2.5 µl of NAD<sup>+</sup> + 17.5 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 PP-16-2 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 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%.*

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 **PARP7** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.
9. Dilute PARP7 enzyme to **0.78 ng/µl** with 1x PARP Assay Buffer (20 µl/well).
10. Initiate the reaction by adding 20 µl of diluted PARP7 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.

	<b>Blank</b>	<b>Positive Control</b>	<b>Test Inhibitor</b>
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 PARP7 (0.78 ng/µl)	-	20 µl	20 µl
<b>Total</b>	<b>50 µl</b>	<b>50 µl</b>	<b>50 µl</b>

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 Ribose-Binding Agent 2-D 1,000-fold with Blocking Buffer 3 (50 µl/well).
2. Add 50 µl of diluted Ribose-Binding Agent 2-D to every well.
3. Incubate 45 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. Dilute 5000-fold Secondary HRP-Antibody 2 in Blocking Buffer 3 (50 µl/well).
6. Add 50 µl of diluted Secondary HRP-Antibody 2 to each well.
7. Incubate for 30 minutes at RT.
8. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.
9. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).
10. Add 100 µl of mix to every well.
11. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.
12. 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

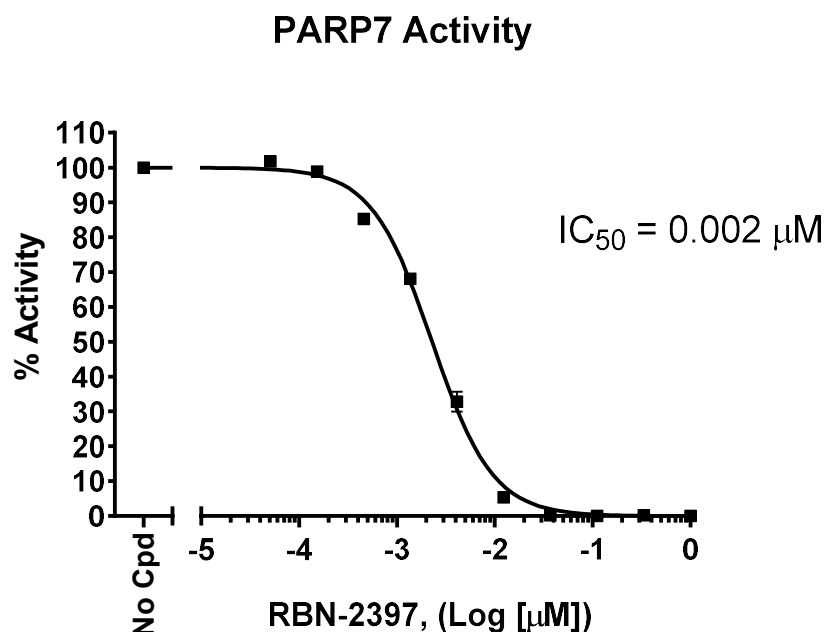


Figure 2: Inhibition of PARP7 activity by RBN-2397.

PARP7 was incubated with increasing concentrations of RBN-2397 (#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](mailto:support@bpsbioscience.com).

## References

Sanderson D., *et al.*, 2023 *Cell Chemical Biology* 30(1): 43-54.

## Troubleshooting Guide

Visit [bpsbioscience.com/assay-kits-faq](https://bpsbioscience.com/assay-kits-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto: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

Version 031924