Description

The PARP3 Homogeneous Assay Kit is designed to measure PARP3 (poly-(ADP-ribose) polymerase 3) activity for screening and profiling applications. The PARP3 Homogeneous Assay Kit comes in a convenient 384-well AlphaLISA® format, with enough purified recombinant PARP3, biotinylated histone substrate, ADP-Ribose Binding Reagent 1 and assay buffer for 400 enzyme reactions.

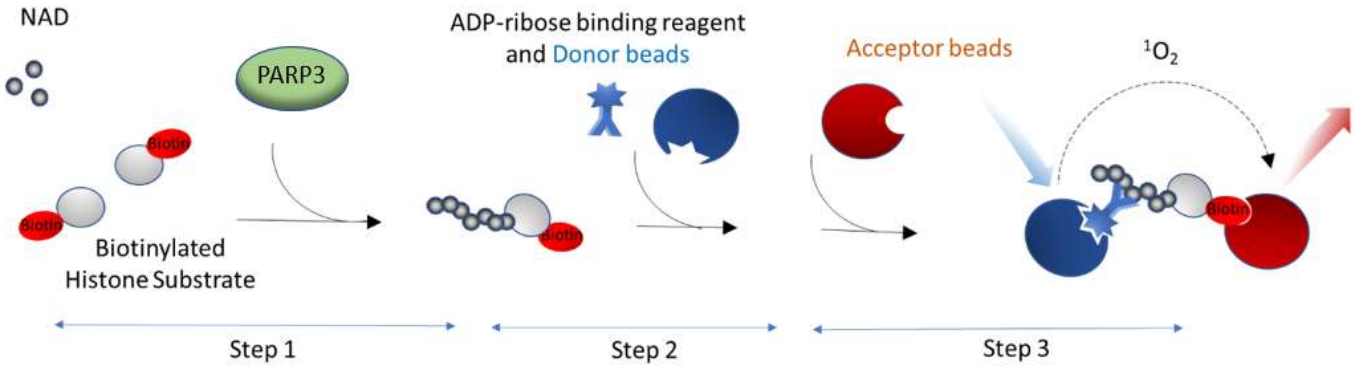

Figure 1: PARP3 Homogenous Assay Kit schematic.

A sample containing PARP3 is incubated with a biotinylated histone substrate and NAD^+ for one hour. This is followed by the addition of acceptor beads and ADP-Ribose Binding Reagent 1, and finally donor beads. Alpha-counts are then measured. Alpha-counts are directly proportional to PARP3 activity.

Background:

PARP3, also known as poly-(ADP-ribose) polymerase 3 or NAD^+ ADP-ribosyltransferase 3, 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. PARP3 is involved in programmed and stress-induced double strand break repair via classical NHEJ (non-homologous end-joining). In the absence of PARP3, DNA repair is less efficient, and cytotoxicity is potentiated when DNA damaging insults occur. It has been linked to cancer, with upregulation of PARP3 occurring in TGFβ (transforming growth factor β)-induced cell EMT (epithelial to mesenchymal transition) in breast cancer. It also promotes cell stemness. The use of inhibitors towards PARP3 can refrain cells from EMT. In addition, the mTORC2 (mTOR complex 2) signaling pathway is impacted, contributing to synthetic lethality in cells deficient in a complementary DNA repair pathway, such as in BRCA1 (breast cancer type 1 susceptibility protein)- triple negative breast cancers (TNBC). PARP3 acts in synergy with PARP1 in the response to ionizing radiation, and the use of inhibitors for both PARP1 and PARP3 also provides an opportunity to reduce the concentration of both and decrease the radiation regimen.

Application(s)

Screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
80503	PARP3, GST-Tag*	5.2 µg	-80°C
	5X PP-01 Assay Buffer	2 x 1 ml	-80°C
	Biotinylated Histone Substrate	500 reactions	-80°C
78311	ADP-Ribose Binding Reagent 1	10 µl	-80°C
	750 µM NAD^+	400 µl	-80°C
	0.5 mM DTT	200 µl	-20°C
	4X Detection Buffer 1NP	2 ml	-20°C

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

Materials Required but Not Supplied

Name	Ordering Information
AlphaLISA® Anti-Rabbit IgG Acceptor Beads	Perkin Elmer #AL104C
AlphaScreen® Streptavidin-Conjugated Donor Beads	Perkin Elmer #6760002S
Optiplate - 384	Perkin Elmer #6007290
AlphaScreen microplate reader	

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 PARP3 Homogenous Assay Kit is compatible with up to 1% final DMSO concentration.
- Avoid green and blue dyes that absorb light in the AlphaScreen signal emission range (λ=520-620 nm), such as Trypan Blue.
- Avoid the use of potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}).
- The presence of >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. Media like MEM, which lacks these components, does not affect AlphaScreen assays.

Assay Protocol

- All samples and controls should be tested in triplicate.
- The assay should include "Blank", "Positive Control" and "Test inhibitor".
- 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 Talazoparib (#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*:*

1. Reconstitute Biotinylated Histone Substrate with 500 µl of distilled water.

   Note: Diluted Biotinylated Histone Substrate can be stored into single use aliquots (minimum volume 5 µl/aliquot) and stored at -80°C.

2. Thaw 5X PP-01 Assay Buffer.

3. Dilute 0.5 M DTT 50-fold with 5X PP-01 Assay Buffer. This makes 5X PP-01 Assay Buffer with 10 mM DTT.

   Note: Prepare only enough DTT-containing buffer as required for the assay. Store the remaining assay buffer at -20°C.

4. Dilute 5x PP-01 Assay Buffer with 10 mM DTT 5-fold with distilled water. This makes 1x PP-01 Assay Buffer.

5. Prepare the Test Inhibitor (3 µl/well): for a titration prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 15 µl.

   5.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in distilled water, 5-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use distilled water (Diluent Solution).

   OR

   5.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 20-fold in distilled water to prepare the highest concentration of the 5-fold intermediate dilutions. The concentration of DMSO is now 5%.

   Prepare serial dilutions of the Test Inhibitor at 5-fold the desired final concentrations using 5% DMSO in distilled water to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 5% 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%.

Step 2:

1. Make a Master Mix (7 µl/ well): N wells × (1 µl of reconstituted Biotinylated Histone Substrate + 1 µl of 750 µM NAD^+ + 2 µl of 5X PP-01 Assay Buffer with 10 mM DTT + 3 µl of distilled water).

2. Add 7 µl of Master Mix to each well.

3. Thaw **PARP3** on ice. Briefly spin the tube containing enzyme to recover the full content of the tube.

4. Dilute PARP3 to 2.6 ng/µl with 1X PP-01 Assay Buffer (5 µl/ well).

5. Add 3 µl of inhibitor solution to each well designated "Test Inhibitor".

6. Add 3 µl of the Diluent Solution to the "Blank" and "Positive Control" wells.

7. Add 5 µl of 1x PP-01 Assay Buffer to the "Blank" wells.

8. Initiate the reaction by adding 5 µl of diluted PARP3 to the wells labeled "Positive Control" and "Test Inhibitor".

9. Incubate at Room Temperature (RT) for 1 hour with slow agitation.

Component	**Test Inhibitor**	**Blank**	**Positive Control**
Master Mix	7 µl	7 µl	7 µl
Test Inhibitor	3 µl	-	–
Diluent Solution	–	3 µl	3 µl
1x PP-01 Assay Buffer	-	5 µl	-
Diluted PARP3 (2.6 ng/µl)	5 µl	-	5 µl
Total	**15 µl**	**15 µl**	**15 µl**

Protect your samples from direct exposure to light for step 3. Photobleaching will occur!

Step 3:

1. Dilute 4-fold the 4X Detection Buffer 1NP with distilled water. This makes 1X Detection Buffer.

2. Prepare a mixture containing AlphaLISA™ Anti-Rabbit Acceptor Beads diluted 250-fold and ADP-Ribose Binding Reagent 1 diluted 400-fold in 1X Detection Buffer (10 µl of mix/well).

3. Add 10 µl of mixture to each well.

4. Briefly agitate the plate.

5. Dilute AlphaScreen® Streptavidin-Conjugated Donor Beads 250-fold with 1x Detection Buffer (10 µl/ well).

6. Add 10 µl per well.

7. Incubate for up to 1 hour at RT.

   Note: First reading can be done 5-15 minutes, but longer incubation (up to 1 hour) is recommended for better readouts.

8. Read Alpha-counts on an AlphaScreen® microplate reader.

9. The "Blank" control might be important to determine the background A-screen counts in the assay. The blank value should be subtracted from all other values.

Example Results

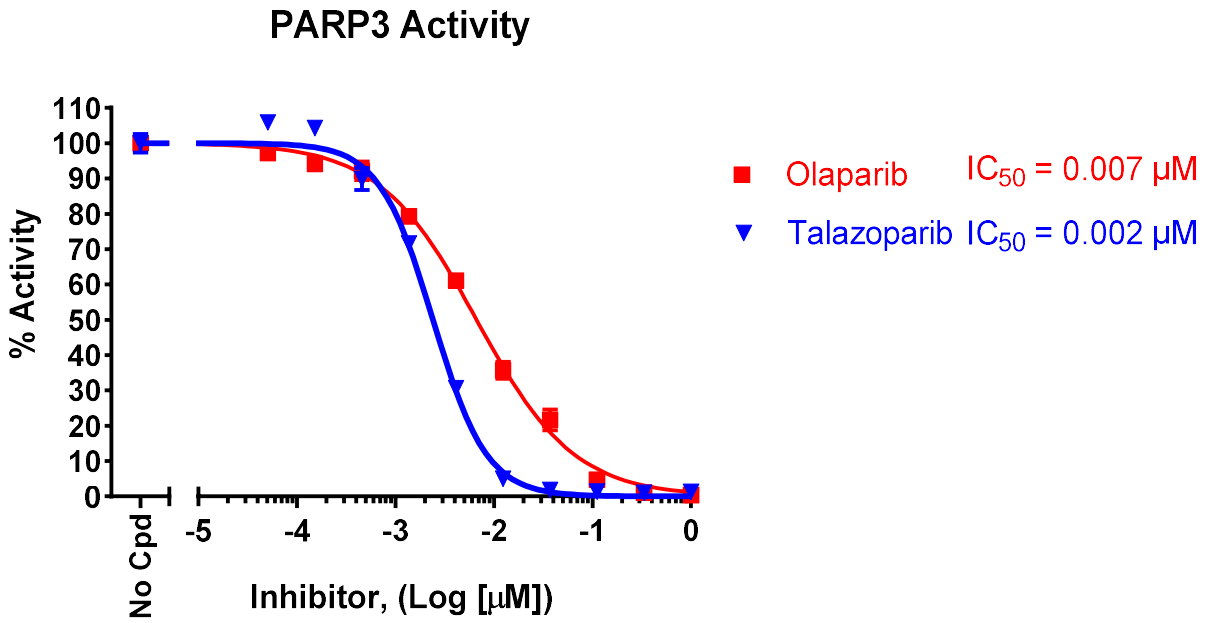

Figure 2: Inhibition of PARP3 activity by Olaparib and Talazoparib.
PARP3 was incubated with increasing concentrations of Olaparib (#78318 or #27003) and Talazoparib (#78318). Results are expressed as percent activity, in which absence of inhibitor is set to 100%.

Data shown is representative. 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

References
Rodriguez-Vargas J., *et al.*, 2019 *Cell Cyle* 18(12): 1295-1301.

Related Products

Products	*Catalog #*	*Size*
PARP1 Homogeneous Assay Kit	78438	384 reactions
PARP2 Homogeneous Assay Kit	78572	384 reactions
PARPtrap™ Assay Kit for PARP1	80584	96 reactions/384 reactions
PARPtrap™ Assay Kit for PARP2	78296	96 reactions/384 reactions
PARPtrap™ Combo Assay Kit for PARP1 and PARP2	78317	384 reactions
TNKS1 Homogeneous Assay Kit	78489	384 reactions

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