Description

The PARP2 Homogeneous Assay Kit is designed to measure PARP2 activity for screening and profiling applications. PARP2 is known to catalyze the NAD-dependent addition of poly(ADP-ribose) to histones. The PARP2 Homogeneous Assay Kit comes in a convenient AlphaLISA® format, with biotinylated histone substrate, recombinant antibody (ADP-ribose binding reagent 1), PP-01 assay buffer, and purified PARP2 for 384 enzyme reactions. The PARP2 Homogeneous Assay Kit takes advantage of a highly specific antibody that recognizes the PARylated form of the substrate. With this kit, only three simple steps are required for PARP2 reactions. First, a sample containing PARP2 enzyme is incubated with biotinylated substrate and NAD for one hour. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

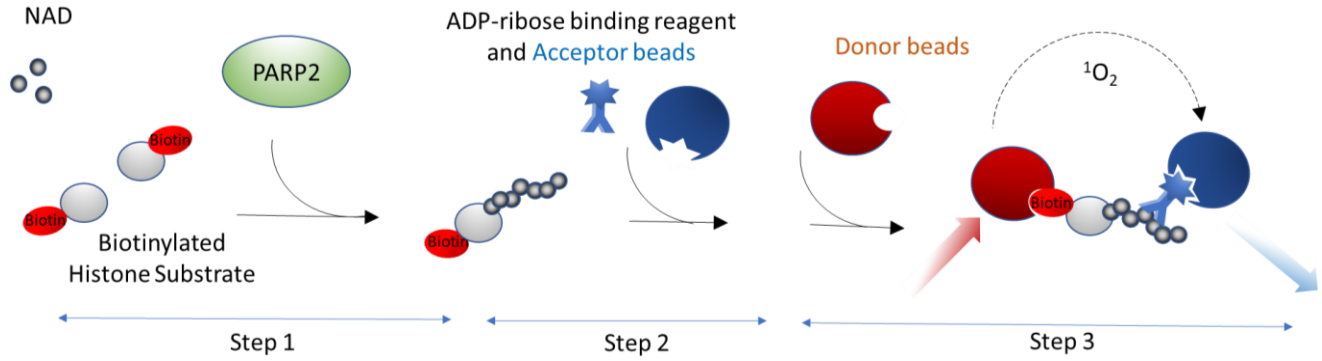

Figure 1. PARP2 Homogenous Assay Kit schematic

Applications

Screen molecules that inhibit PARP1 activity.

Supplied Materials

Catalog #	Name	Amount	Storage	
80502	PARP2, GST-Tag*	8 µg	-80°C	***Avoid multiple freeze/ thaw cycles***
	5X PP-01 Assay Buffer	2 x 1 ml	-80°C	
	Biotinylated histone substrate**	500 rxns	-80°C	
78311	ADP-ribose binding reagent 1	10 µl	-80°C	
	NAD+ (750 µM)	400 µl	-80°C	
52301	4X Detection Buffer 1	2 ml	-80°C	

**The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.*
***Reconstitute in 500 µl H_2O*

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	
0.5 M DTT	

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 PARP2 Homogenous Assay Kit is compatible with up to 1% final DMSO concentration.
We recommend preparing the inhibitor in no higher than 5% DMSO solution in buffer and using 3 µl per well.

Avoid green and blue dyes that absorb light in the AlphaScreen signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the 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. MEM, which lacks these components, does not affect AlphaScreen assays.

Assay Protocol

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

Reagent Preparation

1. Reconstitute the biotinylated histone substrate in 500 µl of distilled water. Aliquot biotinylated histone substrate into single use aliquots depending on how many times the plate will be used. Store aliquots at -80°C.

2. Thaw 5X PP-01 assay buffer. Add 0.5 M DTT to 5X PP-01 assay buffer for a final concentration of 10 mM DTT.

   Note: DTT should be added just before use. Prepare only enough DTT-containing buffer as required for the assay. Store the remaining assay buffer at -20°C.

3. Prepare 1X PP-01 assay buffer by adding 1 part of 5X PP-01 assay buffer **(with DTT)** to 4 parts distilled water.

4. Prepare the compound solution.

   a. 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 20-fold in water.

      Note: To run an IC_{50} or test lower concentrations of the compound, prepare serial dilutions using water containing 5% DMSO, so the final concentration of DMSO will be 1% in all samples.

   b. If the compound is soluble in water, prepare a solution of the compound in water that is 5-fold higher than the final assay concentration.

Reaction Setup

1. Make Master Mix: N wells × (1 µl of **Biotinylated histone substrate** + 1 µl of **NAD+ (750 µM)** + 2 µl of **5x stock Assay Buffer and** + 3 µl of **water**).

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

3. To the wells designated as "Blank", add 5 µl of **1X PP-01 assay buffer** and 3 µl of **diluent solution without inhibitor** (for example DMSO 5%).

Component	**Blank**
Master Mix	7 µl
Diluent solution* (no inhibitor)	3 µl
1X PP-01 Assay Buffer	5 µl
Total	**15 µl**

**The diluent solution contains the water with the same concentration of solvent (e.g. DMSO) as the test compound solution.*

4. Thaw PARP2 on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube.

5. Calculate the amount of enzyme required for your assay and dilute PARP2 in 1X PP-01 assay buffer (**with DTT**) to 4 ng/µl (the final amount of PARP2 in the assay will be 20 ng/rxn). Keep the diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

   Note: Aliquot the remaining undiluted PARP2 enzyme into single use aliquots and store at -80°C.

PARP2 is sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

6. Add 3 µl of inhibitor solution to each well designated "Test Inhibitor". To "Positive Control" add 3 µl of the diluent solution without inhibitor.

7. Initiate the reaction by adding 5 µl of **PARP2** to the wells labeled "Positive Control" and "Test Inhibitor".

Component	**Test Inhibitor**	**Positive Control**
Master Mix	7 µl	7 µl
Test compound	3 µl	–
Diluent solution* (no inhibitor)	–	3 µl
PARP2 (4 ng/µl)	5 µl	5 µl
Total	**15 µl**	**15 µl**

**The diluent solution contains water with the same concentration of solvent (e.g. DMSO) as the test compound solution.*

8. Once the reaction mixture (15 µl) is added to 384-well plate, incubate at room temperature for 1 hour with slow shaking.

Reaction Detection and Reading Results

Protect your samples from direct exposure to light. Photobleaching will occur.

1. Prepare the 1X Detection buffer by adding 1-part 4X Detection buffer to 3 parts distilled water.
2. Prepare a mixture in 1X Detection buffer containing:
    a. anti-Rabbit Acceptor beads (Perkin Elmer #AL104C) diluted 250-fold
    b. ADP-ribose binding reagent 1 diluted 400-fold
3. Add 10 µl of this mixture to each well then briefly shake plate.
4. Dilute the Streptavidin-conjugated donor beads (PE #6760002S) 250-fold with 1x Detection buffer. Add 10 µl per well. Incubate for at least 5-15 min at room temperature.
5. Read Alpha-counts.

Example Results

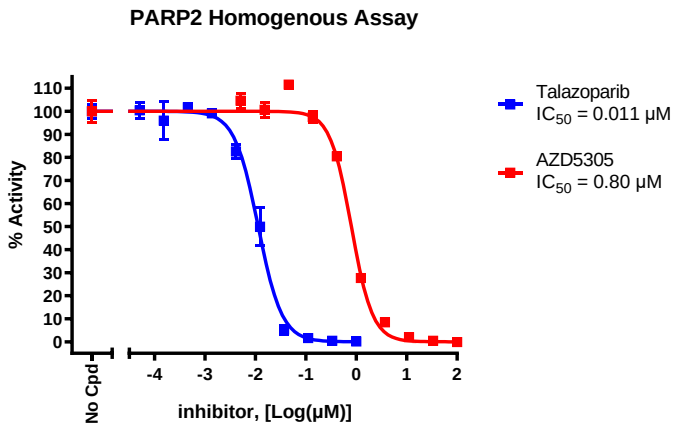

Figure 1: Inhibition of PARP2 activity by Talazoparib (Selleckchem) and AZD5305 (MedChemExpress), measured using the PARP2 Homogenous Assay Kit (BPS Bioscience #78572).

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

Related Products

Products	Catalog #	Size
PARP1 Chemiluminescent Assay Kit	80551	96 rxns
PARP1 Chemiluminescent Assay Kit (384-well)	80569	384 rxns
PARP1 Colorimetric Assay Kit	80580	96 rxns
PARP2 Chemiluminescent Assay Kit	80552	96 rxns/384 rxns
PARP2 Colorimetric Assay Kit	80581	96 rxns
PARP2 Homogeneous Assay Kit	80702	384 rxns
PARP3 Chemiluminescent Assay Kit	80553	96 rxns
PARP6 Chemiluminescent Assay Kit	80556	96 rxns
PARP10 Chemiluminescent Assay Kit	80560	96 rxns
PARP10, FLAG-Strep-Tag Recombinant	80522	10 µg
PARP11 Chemiluminescent Assay Kit	80561	96 rxns
PARP14 Chemiluminescent Assay Kit	80568	96 rxns
PARP15 Chemiluminescent Assay Kit	80567	96 rxns
PARPtrap™ Assay Kit for PARP1	80584	96 rxns
PARPtrap™ Assay Kit for PARP2	78296	96 rxns/384 rxns
PARPtrap™ Combo Assay Kit for PARP1 and PARP2	78317	384 rxns
TNKS1 (PARP5A) Chemiluminescent Assay Kit	78405	96 rxns
TNKS1 Histone Ribosylation Assay Kit (Biotin-labeled NAD+)	80579	384 rxns
TNKS1 Histone Ribosylation Colorimetric Assay Kit	80582	96 rxns
TNKS2 (PARP5B) Chemiluminescent Assay Kit	78406	96 rxns
TNKS2 Histone Ribosylation Assay Kit (Biotin-labeled NAD+)	80572	384 rxns
TNKS2 Histone Ribosylation Colorimetric Assay Kit	80583	96 rxns
Streptavidin-HRP (For PARP & Cytokine Assay Kits)	80611	100 µl