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Data Sheet

PARP1 Colorimetric Assay Kit

Catalog # 80580

DESCRIPTION: The *PARP1 Colorimetric Activity 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 96-well format, with purified PARP1 enzyme, histone mixture, activated DNA, and PARP1 assay buffer for 100 enzyme reactions. The key to the *PARP1 Colorimetric Activity Assay* is the biotinylated substrate. With this kit, only three simple steps are required for PARP1 reactions. First, histone proteins are coated on a 96-well plate. Next, the PARP1 biotinylated substrate is incubated with an assay buffer that contains the PARP1 enzyme. Finally, the plate is treated with streptavidin-HRP followed by addition of the colorimetric HRP substrate to produce color that can then be measured using a UV/Vis spectrophotometer microplate reader.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
80501	PARP1	5 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
52029	5x histone mixture	1 ml	-80°C	
80601	10x assay mixture containing biotinylated substrate	300 µl	-80°C	
80602	10x PARP assay buffer	1 ml	-20°C	
	Blocking buffer	25 ml	+4°C	
	Activated DNA	500 µl	-80°C	
	Streptavidin-HRP	100 µl	+4°C	
	Colorimetric HRP substrate	10 ml	+4°C	
	Transparent 96-well plate	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
1x PBS buffer
PBST buffer (1x PBS, containing 0.05% Tween-20)
2 M sulfuric acid (aqueous)
Rotating or rocker platform
Adjustable micropipettor and sterile tips
UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm*

*Alternately, a spectrophotometer reading at 650 nm may be used, but sensitivity of the assay will be greatly reduced.

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

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STABILITY: Up to 1 year when stored as recommended.

REFERENCE(S): Brown, J.A., Marala, R.B. 2002. *J. Pharmacol. Toxicol. Methods* **47:**137-41.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Step 1: Coat 50 µl of histone solution to the 96-well microtiter plate

1) Dilute **5x histone mixture** 1:5 with 1x PBS.

2) Add 50 µl of histone solution to each well and incubate at 4ºC overnight (or incubate at 30ºC for 90 minutes).

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

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

5) Block the wells by adding 200 µl of **Blocking buffer** to every well. Incubate at room temperature for 60-90 minutes.

6) Wash plate three times with 200 µl PBST buffer as described above.

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

Step 2: Ribosylation reaction

1) Prepare the master mixture: N wells x (2.5 µl 10x PARP buffer + 2.5 µl **10x PARP Assay mixture** + 5 µl **Activated DNA** + 15 µl distilled water). Add 25 µl to every well.

2) Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor”. For the “Positive Control" and “Blank”, add 5 µl of the same solution without inhibitor (Inhibitor buffer). *Note: The PARP1 Colorimetric Assay Kit is compatible with up to 1% final DMSO concentration. We recommend preparing the inhibitor in 10% DMSO aqueous solution and using 5 µl per PARP1 reaction.*

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	Positive Control	Test Inhibitor	Blank
10x PARP buffer	2.5 µl	2.5 µl	2.5 µl
10x Assay mixture	2.5 µl	2.5 µl	2.5 µl
Activated DNA	5 µl	5 µl	5 µl
Distilled water	15 µl	15 µl	15 µl
Test Inhibitor	–	5µl	–
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl
1x PARP buffer	–	–	20 µl
PARP1 (2 - 2.5 ng/µl)	20 µl	20 µl	
Total	50 µl	50 µl	50 µl

3) Prepare **1x PARP buffer** by adding 1 part of **10x PARP buffer** to 9 parts H_2O (v/v). To the wells designated as "Blank", add 20 µl of **1x PARP buffer**.

4) Thaw **PARP1 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **PARP1** required for the assay and dilute enzyme to 2.0 ~ 2.5 ng/µl with **1x PARP buffer**. Aliquot remaining **PARP1 enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **PARP1 enzyme** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

5) Initiate reaction by adding 20 µl of diluted **PARP1 enzyme** to the wells designated “Positive Control” and "Test Inhibitor Control". Incubate at room temperature for 1 hour.

6) Discard the reaction mixture after 1 hour, and wash plate three times with 200 µl PBST buffer and tap plate onto clean paper towel as described above.

Step 3: Detection

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

2) Add 50 µl of diluted **Streptavidin-HRP** to each well. Incubate for 30 min. at room temperature.

3) Wash three times with 200 µl PBST buffer and tap plate onto clean paper towel as described above.

4) Add 100 µl of the **colorimetric HRP substrate** to each well and incubate the plate at the room temperature until blue color is developed in the positive control well. For PARP1, it normally takes 15~20 min to fully develop the color. However, the optimal incubation time may vary, and should be determined empirically by the user.

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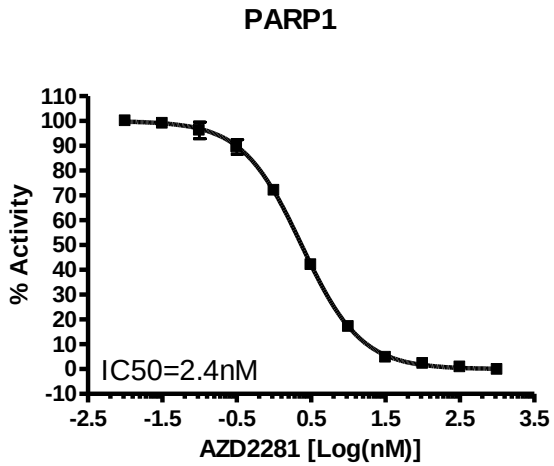
5) After the blue color is developed, add 100 µl of 2 M sulfuric acid to each well. Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader. The negative control-blank well should be ~ 0.05 absorbance at 450 nm. *Alternatively, the plate may be read at 650 nm without adding 2 M sulfuric acid, but the Signal-to-Background ratio will be decreased.*

Example of Assay Results:



PARP1 activity, measured using the *PARP1 Colorimetric Activity Assay Kit* (BPS Cat. #80580), in the presence of the PARP inhibitor, AZD2281 (BPS Cat. #27003). Absorbance at 450 nm was measured using a Tecan microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

Product Name	Catalog #	Size
PARP2 Colorometric Assay Kit	80581	96 rxns
TNKS1 Colorometric Assay Kit	80582	96 rxns
TNKS2 Colorometric Assay Kit	80583	96 rxns
PARP1 Chemiluminescent Assay Kit	80551	96 rxns
PARP2 Chemiluminescent Assay Kit	80552	96 rxns.
PARP3 Chemiluminescent Assay Kit	80553	96 rxns.
PARP5A (TNKS1) Chemiluminescent Assay Kit	80573	96 rxns.
PARP5B (TNKS2) Chemiluminescent Assay Kit	80579	96 rxns.
PARP6 Chemiluminescent Assay Kit	80556	32 rxns.
PARP1 Enzyme	80501	10 µg
PARP2 Enzyme	80502	10 µg
PARP3 Enzyme	80503	10 µg
PARP6 Enzyme	80506	10 µg
TNKS2 (PARP5A) Enzyme	80504	10 µg
TNKS2 (PARP5B/C) Enzyme	80505	10 µg
PARP7 Enzyme	80507	10 µg
PARP9 Enzyme	80509	10 µg
PARP11 Enzyme	80511	10 µg

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Colorimetric signal of positive control reaction is weak	Enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme. Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity.
	Colorimetric HRP substrate was not incubated long enough	Increase the amount of time that the colorimetric HRP substrate is incubated in the wells. Avoid azides.
Colorimetric signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
	Signal is out of range of detection (too high)	Decrease the amount of time that the colorimetric HRP substrate is incubated in the wells
Background (signal to noise ratio) is high	Insufficient washes or blocking	Be sure to include blocking steps after wash steps. Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST. Be sure to dilute Streptavidin-HRP in blocking buffer, not assay buffer.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1%. Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of enzyme to create a standard curve.

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