6405 Mira Mesa Blvd. Suite 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

PARP2 Homogeneous Assay Kit

Catalog # 80702

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, primary antibody, PARP assay buffer, and purified PARP2 for 384 enzyme reactions. The key to the *PARP2 Homogeneous Assay Kit* is a highly specific antibody that recognizes PARylated substrate. With this kit, only three simple steps are required for PARP2 reactions. First, a sample containing PARP2 enzyme is incubated with activated DNA and biotinylated substrate for one hour. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

	Cat. #		Amount	Storage
(Avoid freeze/thaw cycles!)	80502	PARP2	10 µg	-80°C
		NAD+ (750 µM)	400 µl	-20°C
	52140K	Primary antibody 11	10 µl	-80°C
		Biotinylated histone substrate*	500 rxns	-80°C
	80602A	10x PARP assay buffer**	1 ml	-20°C
	52301	4x Detection buffer 1	2 ml	-20°C

*Reconstitute in 500 µl H20
**Add DTT before use

MATERIALS REQUIRED BUT NOT SUPPLIED:
AlphaLISA anti-mIgG acceptor beads, 5 mg/ml (Perkin Elmer #AL105C)
AlphaScreen Streptavidin-conjugated donor beads, 5 mg/ml (Perkin Elmer #6760002S)
Optiplate -384 (Perkin Elmer #6007290)
AlphaScreen microplate reader
Adjustable micropipettor and sterile tips
0.5 M DTT

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

CONTRAINDICATIONS: 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.

6405 Mira Mesa Blvd. Suite 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

STABILITY: At least one year from date of receipt when stored as directed.

REFERENCES:
Brown JA, Marala RB. *J. Pharmacol. Toxicol. Methods* 2002 **47:**137-41.

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

Step 1:
1) Thaw 10x PARP assay buffer. Add 0.5 M DTT to 10x PARP assay buffer to make a final concentration of 10 mM. (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).

2) Reconstitute biotinylated histone substrate in 500 µl distilled water.

3) Thaw PARP2 on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot PARP2 enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PARP2 is sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Prepare 1X PARP assay buffer by adding 1 part PARP assay buffer to 9 parts distilled water.

5) Dilute PARP2 in 1X PARP assay buffer (with DTT) at 4 ng/µl (20 ng/5 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

6) Using master mixes as much as possible, add the following reagents to the microwells, in duplicate:

	Positive Control	**Test Sample**	**Substrate Control**	**Blank**
PARP2 (4 ng/µl)	5 µl	5 µl	5 µl	–
10x PARP assay buffer	1.5 µl	1.5 µl	1.5 µl	1.5 µl
NAD+ (750 µM)	1 µl	1 µl	–	1 µl
Biotinylated substrate	1 µl	1 µl	1 µl	1 µl
Test Inhibitor/Activator	–	X µl	–	–
H_2O	6.5 µl	6.5 – X µl	7.5 µl	11.5 µl
Total	**15 µl**	**15 µl**	**15 µl**	**15 µl**

7) Once entire reaction mixture (15 µl) is added to 384-well plate, Incubate at room temperature for 1 hour with slow shaking

6405 Mira Mesa Blvd. Suite 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Step 2:
Note: Protect your samples from direct exposure to light!

1) Prepare 1x detection buffer by adding 1 part 4x detection buffer to 3 parts distilled water.

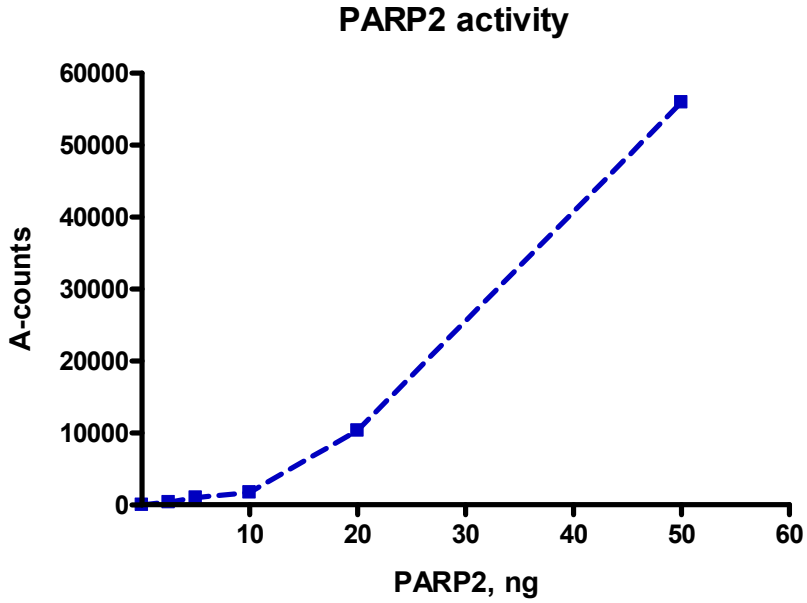
2) Dilute anti-Mouse Acceptor beads (Perkin Elmer #AL105C) 1:125-fold with 1x Detection buffer. Add 5 µl per well. Shake plate briefly.

3) Dilute "Primary antibody 11" 200-fold with 1x Detection buffer. Add 5 µl per well. Shake plate. Incubate 30 min at room temperature with slow shaking

Step 3:

1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with 1x Detection buffer. Add 10 µl per well. Incubate for 60-90 min at room temperature.
2) Read Alpha-counts.

Example of Assay Results:



PARP2 enzyme activity, measured using the PARP2 Homogeneous Assay Kit, BPS Bioscience Cat. #80702. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

6405 Mira Mesa Blvd. Suite 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

RELATED PRODUCTS:

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	#80564	96 rxns.
PARP5B (TNKS2) Chemiluminescent Assay Kit	#80566	96 rxns.
PARP6 Chemiluminescent Assay Kit	#80556	32 rxns.
PARP7 Chemiluminescent Assay Kit	#80557	96 rxns.
PARP11 Chemiluminescent Assay Kit	#80561	96 rxns.
PARP1 Enzyme	#80501	10 µg
PARP2 Enzyme	#80502	10 µg
PARP3 Enzyme	#80503	10 µg
PARP6 Enzyme	#80506	10 µg
TNKS1 (PARP5A) Enzyme	#80504	10 µg
TNKS2 (PARP5B/C) Enzyme (667-end)	#80505	10 µg
TNKS2 (PARP5B/C) Enzyme (849-end)	#80515	10 µg
PARP7 Enzyme	#80507	10 µg
PARP9 Enzyme	#80509	10 µg
PARP11 Enzyme	#80511	10 µg
PARP12 Enzyme	#80512	10 µg