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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, activated DNA, 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
LotXXX ***(Avoid freeze/thaw cycles!)***	*80502*	*PARP2*	*30 µg*	*-80°C*
		NAD+	*400 µl*	*-20°C*
		Activated DNA	*600 µl*	*-80°C*
	52140K	*Primary antibody 11*	*40 µl*	*-80°C*
		Biotinylated histone substrate	*400 µl*	*-80°C*
		10x PARP assay buffer	*1 ml*	*-20°C*
		4x Detection buffer	*2 ml*	*-20°C*

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

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.*

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

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

1) 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.

2) Dilute PARP2 in 1X PARP assay buffer at 10-15 ng/µl (50-75 ng/5 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

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

	Positive Control	Test Sample	Substrate Control	Blank
PARP2 (10-15 ng/µl)	*5 µl*	*5 µl*	*5 µl*	*–*
10x PARP assay buffer	*1.5 µl*	*1.5 µl*	*1.5 µl*	*1.5 µl*
Activated DNA	*1.5 µl*	*1.5 µl*	*1.5 µl*	*1.5 µl*
NAD+	*1 µl*	*1 µl*	*–*	*1 µl*
Biotinylated substrate	*1 µl*	*1 µl*	*1 µl*	*1 µl*
Test Inhibitor/Activator	*–*	*X µl*	*–*	*–*
H_2O	*5 µl*	*5 – X µl*	*6 µl*	*10 µl*
Total	**15 µl**	**15 µl**	**15 µl**	**15 µl**

4) Add the entire reaction mixture (15 µl) to the well of a 384-well white plate. Incubate at room temperature for 1 hour.

Step 2:

Note: Protect your samples from direct exposure to light!

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

2) Dilute "Primary antibody 11" 50-fold with 1x Detection buffer. Add 5 µl per well. Shake plate. Incubate 30 min at room temperature.

Step 3:

1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with 1x Detection buffer. Add 10 µl per well. Incubate for 15-20 min. at room temperature.

2) Read Alpha-counts.

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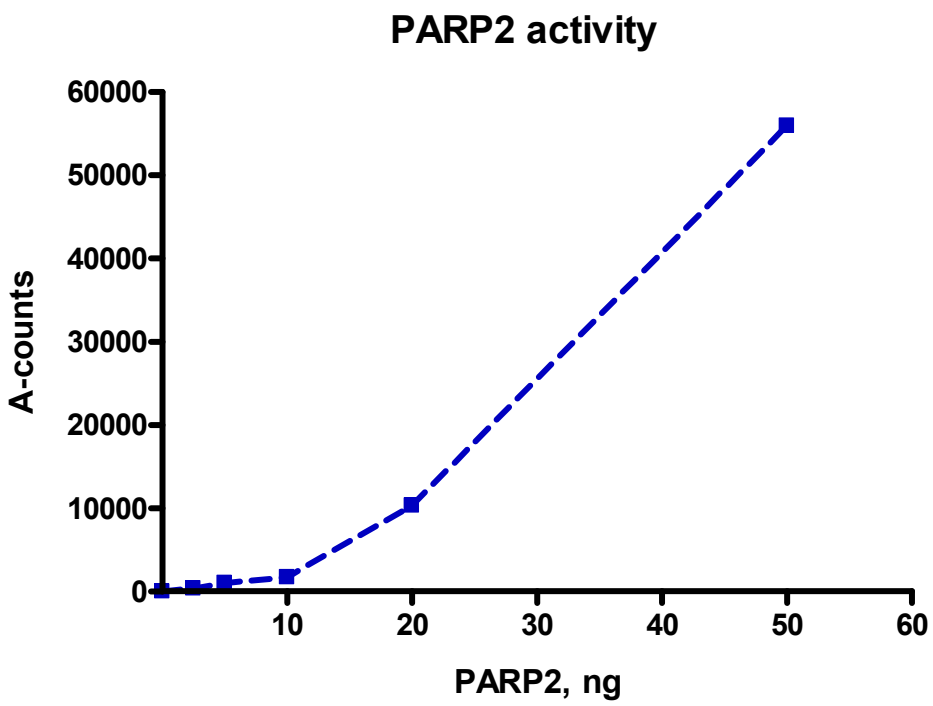
Example of Assay Results:



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

REFERENCES:

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

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*

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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*