

Data Sheet
PARP2 Chemiluminescent Assay Kit
Catalog # 80552

DESCRIPTION: The *PARP2 Chemiluminescent Activity Assay Kit* is designed to measure PARP2 activity using purified PARP2 or cell extracts containing PARP2. PARP2 is known to catalyze the NAD-dependent addition of poly(ADP-ribose) to histones. The PARP2 assay kit comes in a convenient 96-well format, with purified PARP2 enzyme, histone mixture, activated DNA, and PARP2 assay buffer for 100 enzyme reactions. The key to the *PARP2 Chemiluminescent Activity Assay* is the biotinylated substrate. With this kit, only three simple steps are required for PARP2 reactions. First, histone proteins are coated on a 96-well plate. Next, the PARP2 biotinylated substrate is incubated with an assay buffer that contains the PARP2 enzyme. Finally, the plate is treated with streptavidin-HRP followed by addition of the HRP substrate to produce chemiluminescence that can then be measured using a chemiluminescence reader.

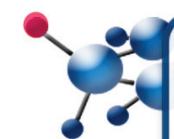
COMPONENTS:

Reagent	Amount	Storage
PARP2 (Code # 80502)	5 µg	-80°C
5x histone mixture	1 ml	-80°C
10x assay mixture containing biotinylated substrate	300 µl	-80°C
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
HRP chemiluminescent substrate (2 components)	6 ml each	+4°C
96-well module	1	Room Temperature

MATERIALS REQUIRED BUT NOT SUPPLIED:

1x PBS buffer
 PBST buffer (1x PBS, containing 0.05% Tween20)
 Luminometer or fluorescent microplate reader capable of reading chemiluminescence
 Adjustable micropipettor and sterile tips
 Rotating or rocker platform
 Paper towels

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

STABILITY: Up to 1 year when stored as recommended.

REFERENCE:

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

Assay Protocol:

Step 1: Coat 50 µl of histone solution to a 96-well module (VWR catalog no. 62409-300)

1. Dilute 5x histone mixture 1:5 with PBS.
2. Add 50 µl of histone solution to each well and incubate at 4°C overnight.
3. Wash the plate three times with PBST buffer (1x PBS containing 0.05% Tween 20).
4. Block the wells by adding 150 µl of Blocking buffer to every well. Incubate at room temperature for 30 min.
5. Wash plate three times with PBST buffer as above.

Step 2: Ribosylation reaction

1. Prepare 1x PARP buffer by adding 1 part of 10x PARP buffer to 9 parts H₂O (v/v)
2. Thaw PARP2 enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of PARP2 required for the assay and dilute enzyme to 2 ng/µl with 1x PARP buffer. Aliquot remaining PARP2 enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PARP2 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

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- Using master mixes as much as possible, add the following reaction mixtures (50 μ l) to the histone-coated 96-well module:

	Positive Control	Sample	Substrate Control	Blank
PARP2 (2 ng/ μ l)	25 μ l		25 μ l	–
Test sample (in 1X PARP buffer)	–	25 μ l	–	–
10x PARP buffer	2.5 μ l	2.5 μ l	2.5 μ l	5 μ l
10x Assay mixture	2.5 μ l	2.5 μ l	–	2.5 μ l
Activated DNA	5 μ l	5 μ l	5 μ l	5 μ l
H ₂ O	15 μ l	15 μ l	17.5 μ l	37.5 μ l
Total	50 μl	50 μl	50 μl	50 μl

- Incubate at room temperature for 1 hour.
- Wash plate three times with PBST buffer as above.

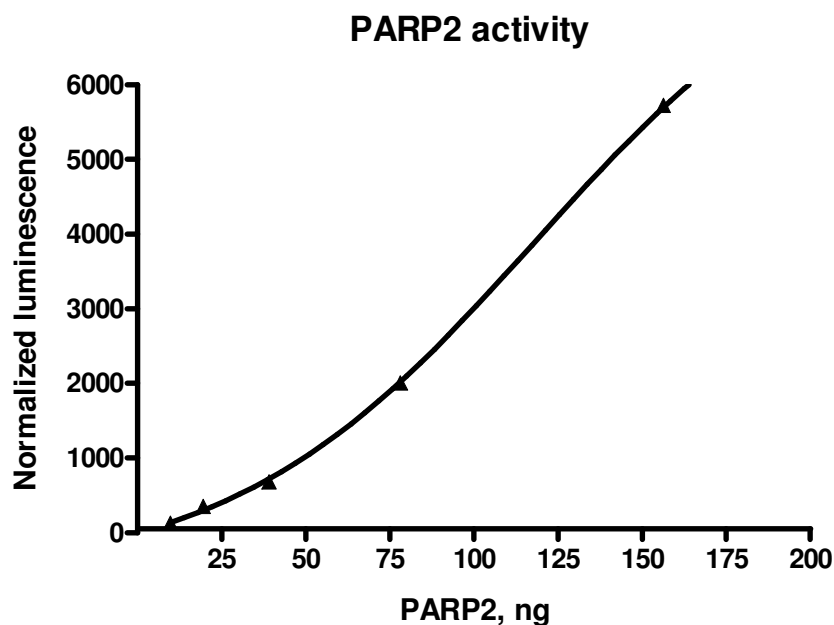
Step 3: Detection

- Dilute Streptavidin-HRP 1:50 in Blocking buffer.
- Add 50 μ l of diluted Streptavidin-HRP to each well. Incubate for 30 min. at room temperature.
- Wash three times with PBST buffer as above.
- Just before use, mix on ice 50 μ l HRP chemiluminescent substrate A and 50 μ l HRP chemiluminescent substrate B and add 100 μ l per well.

Step 4:

Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence.

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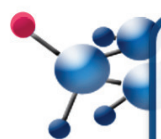
Example of Assay Results:

PARP2 activity, measured using the *PARP2 Chemiluminescent Activity Assay Kit*, Catalogue Code # 80552. Luminescence was measured using a Bio-Tek microplate reader. Data shown is lot-specific. For lot-specific information, please contact amsbio.

RELATED PRODUCTS:

PARP1 Assay Kit	#80551	96 rxns.
PARP3 Assay Kit	#80553	96 rxns.
PARP5b (TNKS2) Assay Kit	#80565	96 rxns.
PARP6 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 (PARP5B/C) Enzyme	#80505	10 µg
PARP7 Enzyme	#80507	10 µg
PARP9 Enzyme	#80509	10 µg
PARP11 Enzyme	#80511	10 µg
PARP12 Enzyme	#80512	10 µg

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