Data Sheet

PARP14 Chemiluminescent Assay Kit

Catalog # AMS.80568
Size: 32 reactions

DESCRIPTION: The *PARP14 Chemiluminescent Activity Assay Kit* is designed to measure PARP14 activity for screening and profiling applications. PARP14 catalyzes NAD-dependent ADP-ribosylation, and PARP14 overexpression in myeloma cells is associated with disease progression and poor survival rates. The PARP14 assay kit comes in a convenient 96-well format, with purified PARP14 enzyme, histone mixture, and PARP assay buffer for 32 enzyme reactions. The key to the *PARP14 Chemiluminescent Activity Assay* is the biotinylated substrate. With this kit, only three simple steps are required for PARP14 reactions. First, histone proteins are coated on a 96-well plate. Next, the biotinylated substrate is incubated with an assay buffer that contains the PARP14 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:

Catalog #	Reagent	Amount	Storage	
80514	PARP14	10 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
52029	5x histone mixture	1 ml	-80°C	
	Opti-PARP assay mixture containing biotinylated substrate	300 µl	-80°C	
80602	10x PARP assay buffer	1 ml	-20°C	
	Blocking buffer	25 ml	+4°C	
	Streptavidin-HRP	100 µl	+4°C	
	HRP chemiluminescent substrate A (translucent bottle)	6 ml	+4°C	
	HRP chemiluminescent substrate B (brown bottle)	6 ml	+4°C	
	Max 96-well module plate	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
1x PBS buffer
PBST buffer (1x PBS, containing 0.05% Tween-20)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

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

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

Step 1: Coat 50 µl of histone solution to a Max 96-well module

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

2) Add 50 µl of histone solution to each well and incubate at 4ºC overnight.

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 1x PARP buffer by adding 1 part of **10x PARP assay buffer** to 9 parts H_2O (v/v)

2) Thaw **PARP14** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of PARP14 required for the assay and dilute enzyme to 10-15 ng/µl with 1x PARP buffer. Aliquot remaining PARP14 enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PARP14 enzyme is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Prepare the master mixture: N wells x (2.5 µl **10x PARP buffer** + 2.5 µl **Opti-PARP Assay mixture** + 20 µl water). Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
10x PARP buffer	2.5 µl	2.5 µl	2.5 µl
Opti-PARP Assay mixture	2.5 µl	2.5 µl	2.5 µl
Water	20 µl	20 µl	20 µl
Test Inhibitor	–	5µl	–
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl
1x PARP buffer	–	–	20 µl
PARP14 (10-15 ng/µl)	20 µl	20 µl	
Total	50 µl	50 µl	50 µl

4) 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 PARP14 Chemiluminescent Assay Kit is compatible with up to 1% final DMSO concentration. It is recommended to prepare the inhibitor in a 10% DMSO aqueous solution and use 5 µl per PARP14 reaction.*

5) To the wells designated as "Blank", add 20 µl of 1X PARP buffer.

6) Initiate reaction by adding 20 µl of diluted PARP14 enzyme to the wells designated "Positive Control" and "Test Inhibitor Control". Incubate at room temperature for 1 hour.

7) 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 above as described above.

4) Just before use, mix on ice 50 µl HRP chemiluminescent substrate A and 50 µl HRP chemiluminescent substrate B and add 100 µl per well.

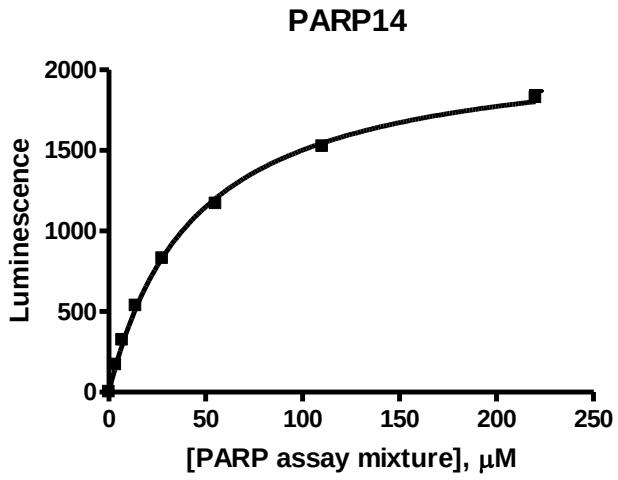
5) Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence. The "Blank" value is subtracted from all other values.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavenlength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example of Assay Results:



PARP14 activity, measured using the *PARP14 Chemiluminescent Activity Assay Kit,* Cat. # AMS.80568. Luminescence was measured using a Bio-Tek microplatereader. *Data shown is lot-specific. For lot-specific information, please contact info@amsbio.com*

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	PARP14 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (PARP14, #80514). 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 of light detection. See section on “Reading Chemiluminescence” above.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent 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.
Background (signal to noise ratio) is high	Insufficient washes	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.
	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 (PARP14, #80514) to create a standard curve.