Data Sheet

PARP1 Chemiluminescent Assay Kit

Catalog # AMS.80551

DESCRIPTION: The *PARP1 Chemiluminescent 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 Chemiluminescent 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 HRP substrate to produce chemiluminescence that can then be measured using a chemiluminescence 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	
80605	Activated DNA (5x)	500 µl	-80°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	
	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.

STABILITY: Up to 1 year when stored as recommended.

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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 96-well module (VWR catalog no. 62409-300)

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

2) Add 50 µl of **histone mixture** 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 buffer** to 9 parts H_2O (v/v)

2) 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.*

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

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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 (5x)	5 µl	5 µl	5 µl
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

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 PARP1 Chemiluminescent 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.*

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

6) 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.

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.

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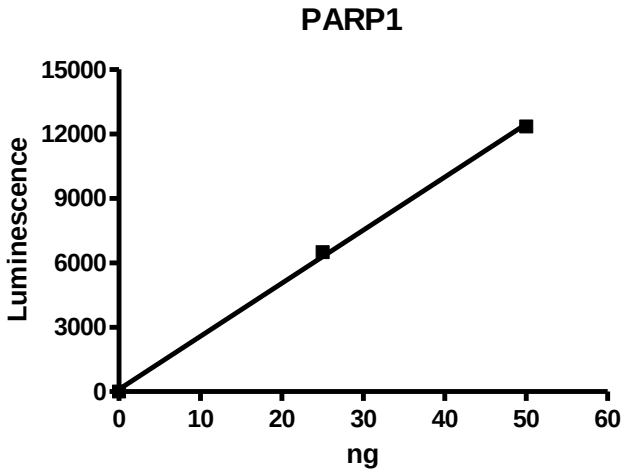
Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength 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:



PARP1 activity, measured using the *PARP1 Chemiluminescent Activity Assay Kit,* Cat. # AMS.80551. Luminescence was measured using a Bio-Tek microplate reader. *Datashown 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	PARP1 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (PARP1, #80501). 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 (PARP1, #80501) to create a standard curve.