CREATIVE BIOMART®
Assay Kit

PARP1 Chemiluminescent Assay Kit

Product Information

Common Name

PARP1

Cat.No. Kit-1792

Product Overview

PARP1 is known to catalyze the NAD-dependent addition of poly (ADP-ribose) to histones.

Description

The PARP1 Chemiluminescent Activity Assay kit is designed to measure PARP1 activity for screening and profiling applications. 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 the assay buffer that contains the PARP1 enzyme. Finally, the plate is treated with streptavidin-HRP followed by addition of the HRP substrate to produce a chemiluminescence that can then be measured using a chemiluminescence reader.

Applications

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

Notes

DMSO >1%, strong acids or bases, ionic detergents, high salt

Storage

12 months from date of receipt, when stored as directed. Kit components require different storage conditions. Be sure to store each component at the proper temperature upon arrival.

Warning

Avoid freeze/thaw cycles.

Synonyms

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poly (ADP-ribose) polymerase 1; poly(ADP-ribosyl)transferase; ADPRT; PARP1

References

Brown, J.A., Marala, R.B., J. Pharmacol. Toxicol. Methods. 2002;47:137-41.
Application Reference(s):
1. Dichamanetin inhibits cancer cell growth by affecting ROS-related signaling components through mitochondrial-mediated apoptosis (2013)
2. Structural basis and SAR for G007-LK, a lead stage 1,2,4-triazole based specific tankyrase 1/2 inhibitor (2013)

Size

96 reactions

Kit Components

PARP1: 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 (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 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

Assay Protocol

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Step 1: Coat 50 µl of histone solution to a 96-well module
1) Dilute 5x histone mixture 1:5 with PBS.
2) Add 50 µl of histone mixture to each well and incubate at 4°C overnight (or incubate at 30°C for 90 minutes).
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 H2O (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.
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

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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 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).

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