SZABO SCANDIC

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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The full length PARP15 (PARP15-FL) Chemiluminescent Assay Kit is designed to measure PARP15 activity for screening and profiling applications. PARP15 is known to catalyze the NAD-dependent ADP-ribosylation of histones. The PARP15-FL assay kit comes in a convenient 96-well format, with purified full length PARP15 enzyme, histone mixture, and PARP assay buffer for 100 enzyme reactions. This Assay Kit takes advantage of the sensitive detection of biotinylated NAD+. First, histone proteins are coated on a 96-well plate. Next, a biotinylated NAD+ mix (termed PARP Substrate Mixture) is incubated with the PARP15-FL enzyme in an optimized assay buffer. Finally, the plate is treated with streptavidin-HRP followed by addition of the ELISA ECL substrate to produce chemiluminescence that can be measured using a chemiluminescence reader.

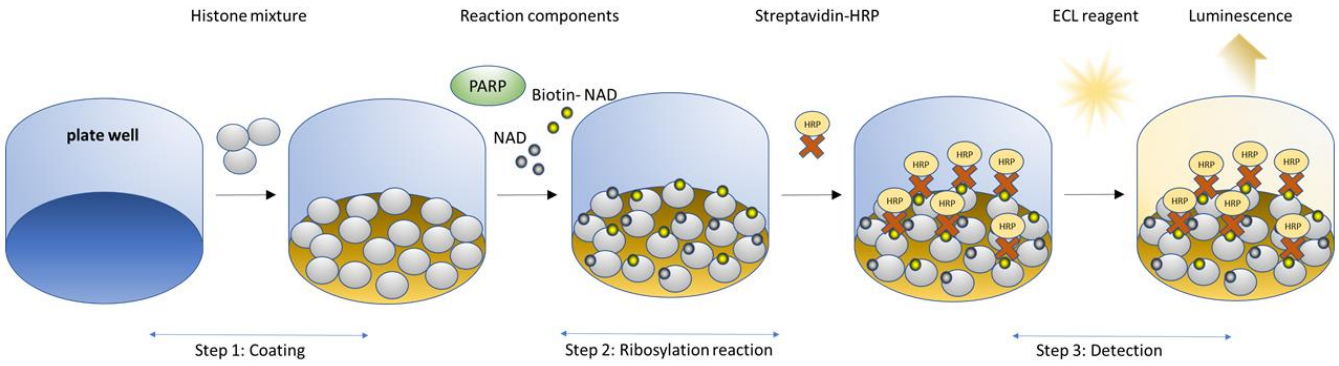

Illustration 1: PARP15-FL Chemiluminescent Assay Kit schematic

Application(s)

Study enzyme kinetics and screen small molecular inhibitors for drug discovery and high throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage	
100191	Full Length PARP15 (His-GST-TEV-2-444e)*	2 µg	-80°C	
52029	5x histone mixture	1 ml	-80°C	
78371	PARP Substrate Mixture 2	250 µl	-80°C	***Avoid multiple freeze/ thaw cycles***
80602	10x PARP assay buffer	1 ml	-20°C	
79743	Blocking buffer 3	25 ml	+4°C	
80611	Streptavidin-HRP	100 µl	+4°C	
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp	
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp	
79837	96-well module plate		Room Temp	

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- DTT (10 mM in water, prepared fresh)
- 1x PBS (phosphate buffer saline) 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

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed. ***Avoid multiple freeze/thaw cycles!***

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The PARP15-FL Chemiluminescent Assay Kit is compatible with up to 1% final DMSO concentration. We recommend preparing the inhibitor in no higher than 10% DMSO solution in buffer and using 5 µl per well.

Assay Protocol

- All samples and controls should be performed in duplicates
- The assay should include a "Blank" and a "Positive control"

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 to make 1x histone mixture.

2) Add 50 µl of histone mixture to each well and incubate at 4°C overnight.

3) Wash the plate three times using 200 µl of PBST buffer (1x PBS containing 0.05% Tween 20) per well.

4) Tap the plate onto clean paper towels to remove the liquid.

5) Block the wells by adding 200 µl of Blocking buffer 3 to every well. Incubate at room temperature for at least 90 minutes.

6) Wash the plate three times with 200 µl of PBST buffer.

7) Tap the plate onto clean paper towels to remove the liquid.

Step 2: Ribosylation reaction

1) Prepare a fresh solution of 10 mM DTT in water.

2) Prepare the Master Mix (25 µl/well): N wells x (2.5 µl of 10x PARP buffer + 2.5 µl of PARP Substrate Mixture 2 + 17.5 µl of water + 2.5 µl of 10 mM fresh DTT).

   Note: the concentration of DTT in the Master Mix will be 1 mM.

3) Add 25 µl of Master Mix to every well.

4) Prepare 1x PARP buffer with DTT: Dilute 10x PARP assay buffer to 1x PARP assay buffer containing DTT by adding 1 volume of 10x PARP assay buffer + 1 volume of 10 mM DTT + 8 volumes of water.

   Note: the concentration of DTT in the 1x PARP assay buffer will be 1 mM

5) Prepare the Test Inhibitor (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   a) If the Test Inhibitor is water-soluble, prepare serial dilutions in 1x PARP buffer (containing DTT), at concentrations 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use 1x PARP buffer containing DTT (Diluent Solution).

   OR

   b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x PARP buffer containing DTT to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   c) Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x PARP buffer (containing DTT) to keep the concentration of DMSO constant.

   d) For positive and blank controls, prepare 10% DMSO in 1x PARP buffer containing DTT (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

6) Add 5 µl of Test Inhibitor to each well labeled as “Test Inhibitor.”
   For the “Positive Control" and “Blank,” add 5 µl of the diluent solution.

   Note: The PARP15-FL Chemiluminescent Assay Kit is compatible with up to 1% final DMSO concentration.

7) Thaw PARP15-FL enzyme on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube. Calculate the amount of PARP15-FL required for the assay and dilute enzyme to **1.0 ng/µl** with 1x PARP buffer with DTT. The final concentration of PARP15-FL will be 5 nM. Aliquot the remaining undiluted PARP15-FL enzyme into aliquots and store at -80°C. Do not re-use these aliquots more than once.

   Note: PARP15-FL enzyme is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. ***Do not re-use the diluted enzyme.***

8) Initiate the reaction by adding 20 µl of diluted PARP15-FL enzyme to the wells designated “Positive Control” and "Test Inhibitor."

9) To the wells designated as "Blank," add 20 µl of 1x PARP buffer with DTT.

   Incubate at room temperature for 1 hour.

	Blank	Positive Control	Test Inhibitor
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x PARP buffer with DTT	20 µl	-	-
PARP15-FL (1.0 ng/µl)		20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

10) Wash the plate three times with 200 µl of PBST buffer and tap the plate onto clean paper towels.

Step 3: Detection

1) Dilute Streptavidin-HRP 1:50 in Blocking buffer 3.

2) Add 50 µl of diluted Streptavidin-HRP to each well. Incubate for 30 minutes at room temperature.

3) Wash three times with 200 µl of PBST buffer and tap the plate onto clean paper towel.

4) Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B. 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).

Example Results

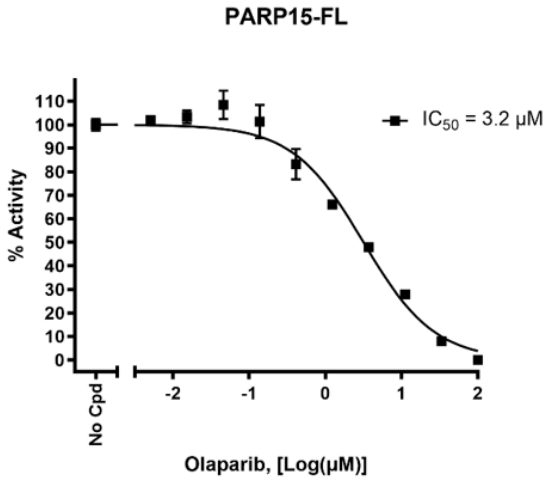

Figure 1: Full length PARP15 activity in the presence of increasing concentrations of Olaparib.
The effect of Olaparib (LC Labs #O-9021) was measured using the PARP15-FL Chemiluminescent Assay Kit (BPS Bioscience #78596). Luminescence was measured using a Bio-Tek microplate reader.

For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
PARP1 Chemiluminescent Assay Kit	80551	96 reactions
PARP2 Chemiluminescent Assay Kit	80552	96 reactions
PARP3 Chemiluminescent Assay Kit	80553	96 reactions
PARP6 Chemiluminescent Assay Kit	80556	96 reactions
PARP7 Chemiluminescent Assay Kit	79729	96 reactions
PARP10 Chemiluminescent Assay Kit	80560	96 reactions
PARP11 Chemiluminescent Assay Kit	80561	96 reactions
PARP14 Chemiluminescent Assay Kit	80568	96 reactions
PARP15 Chemiluminescent Assay Kit	80567	96 reactions
TNKS1 Histone Ribosylation Assay Kit (Biotin-labeled NAD+)	80573	96 reactions
TNKS2 Histone Ribosylation Assay Kit (Biotin-labeled NAD+)	80578	96 reactions