

HT Universal Chemiluminescent PARP Assay Kit with Histone-Coated Strip Wells

Catalog Number: 4676-096-K

Chemiluminescent assay kit for candidate inhibitor screening and determination of IC_{50} values of PARP inhibitors.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Poly ADP-ribosylation of nuclear proteins is a post-translational event that occurs in response to DNA damage. Poly (ADP-ribose) polymerase (PARP) catalyzes the NAD-dependent addition of poly (ADP-ribose) to itself and adjacent nuclear proteins such as histones. PARP contributes to the sequence of events that occurs during DNA base excision repair (1). Whereas PARP-mediated induction of necrosis can occur by extensive depletion of the intracellular NAD pool, (2) the cleavage of PARP-1 promotes apoptosis by preventing DNA repair-induced survival and blocking energy depletion-induced necrosis (3). Experimental models have shown that PARP inhibition prevents tissue damage in animal models of myocardial and neuronal ischemia, diabetes, septic shock, and vascular stroke (4-11). PARP inhibition promotes chemosensitization and radiosensitization of tumors (12).

DESCRIPTION

HT Universal 96-well PARP Assay Kits measure the incorporation of biotinylated poly (ADP-ribose) onto histone proteins in a strip well format. This assay is ideal for screening PARP inhibitors and determining IC_{50} values. Important features of the assay include:

- Chemiluminescent, non-radioactive format.
- Higher throughput 96 test size.
- Sensitivity down to 0.01 Units of PARP per well.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not mix or substitute reagents with those from other lots or sources.
- Variations in sample collection, processing, and storage may cause sample value differences.

TECHNICAL HINTS

- When mixing or reconstituting solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

PRECAUTIONS

The acute and chronic effects of overexposure to reagents of this kit are unknown. Safe laboratory procedures should be followed and protective clothing should be worn when handling kit reagents.

MATERIALS PROVIDED & STORAGE CONDITIONS

Use within 3 months from date of receipt.

| PART | PART # | AMOUNT PROVIDED | STORAGE OF OPENED MATERIAL |
|---|-------------|-----------------------|----------------------------|
| *Histone-Coated Clear Strip Wells | 4678-096-P | 96 wells | Store at 2-8 °C. |
| 10X Strep-Diluent | 4671-096-04 | 2 mL | |
| Strep-HRP | 4800-30-06 | 30 µL | |
| PeroxyGlow™ A | 4675-096-01 | 6 mL | |
| PeroxyGlow™ B | 4675-096-02 | 6 mL | |
| PARP-HSA, 10 U/µL | 4668-050-01 | 50 µL | Store at ≤ -20 °C. |
| *10X Activated DNA | 4671-096-06 | 300 µL | |
| *20X PARP Buffer | 4671-096-02 | 2 vials (500 µL/vial) | Store at ≤ -80 °C. |
| *10X PARP Cocktail <i>Contains biotinylated NAD</i> | 4671-096-03 | 300 µL | |
| *200 mM 3-Aminobenzamide | 4667-50-03 | 60 µL | |
| Plate Sealers | N/A | 4 Adhesive strips | Room temperature |

*Can be stored at ≤ -20 °C for one year in a manual defrost freezer.

OTHER MATERIALS REQUIRED

Reagents:

- Inhibitors
- Phosphate Buffered Saline (PBS)
- PBS + 0.1% Triton X-100
- Distilled water

Equipment:

- Pipettes and pipette tips
- Multichannel pipette 10-200 µL
- Microplate reader capable of measuring absorbance of 450 nm
- Squirt bottle, manifold dispenser, or automated microplate washer

REAGENT PREPARATION

1X Strep-Diluent - Dilute 10X Strep-Diluent 1:10 with distilled water before use. This solution is used as a diluent for the Strep-HRP.

20X PARP Buffer - Dilute the 20X PARP Buffer 1:20 with distilled water. The 1X PARP Buffer is used to rehydrate the histone coated wells, and to dilute the enzyme, PARP Cocktail, and inhibitors.

1X PARP Cocktail - Prepare the 1X PARP Cocktail as follows:

| Reaction Component | Volume |
|--------------------|------------------|
| 10X PARP Cocktail | 2.5 μ L/well |
| 10X Activated DNA | 2.5 μ L/well |
| 1X PARP Buffer | 20 μ L/well |

PARP-HSA Enzyme - The kit contains 50 μ L of PARP-HSA (10 U/ μ L) enzyme. The enzyme should be diluted appropriately with 1X PARP Buffer just before use.

Note: *Diluted enzyme should be used immediately and any remainder discarded.*

PARP Inhibitors - 3-Aminobenzamide (3-AB) is provided at 200 mM in ethanol as a control inhibitor. 3-AB will inhibit the activity of PARP at a wide range of concentrations from 2-10 mM. Serially dilute the stock 3-AB or your PARP inhibitor(s) with 1X PARP Buffer and add to designated wells.

Strep-HRP Working Solution - Just before use, dilute Strep-HRP (10 U/ μ L) 1:500 with 1X Strep-Diluent. A total of 50 μ L/well of Strep-HRP Working Solution is required in the assay.

PeroxyGlow™ A and B Chemiluminescent Substrates - Just before use mix equal volumes of PeroxyGlow A and B together. PeroxyGlow A and B are Horseradish Peroxidase (HRP) substrates generating light that can be quantified in a suitable chemiluminescent strip wells reader.

ASSAY PROTOCOL

RIBOSYLATION REACTION

Note: Do not premix the PARP-HSA Enzyme and the PARP Cocktail since 1X PARP Cocktail will autoribosylate in the presence of NAD.

1. Remove strip wells from the wrapper and add 50 μL /well of 1X PARP Buffer to rehydrate the histones. Incubate at room temperature for 30 minutes. Remove the 1X PARP Buffer from the wells by tapping the strip wells on paper towels.
2. Add serial dilutions of an inhibitor of interest to appropriate wells.
3. Add diluted PARP-HSA enzyme (0.5 U/well) to the wells containing inhibitor. Incubate for 10 minutes at room temperature.
4. Prepare Controls:
 - i. **Negative Control:** A negative control without PARP-HSA enzyme should be prepared to determine background absorbance.
 - ii. **Activity Control for PARP Inhibitor Study:** 0.5 Unit/well PARP-HSA enzyme without inhibitors. These wells provide the 100% activity reference point.
 - iii. **Optional PARP Standard Curve:** Dilute the PARP-HSA Enzyme in cold microtubes with 1X PARP Buffer such that the total activity is 1 U/25 μL , 0.5 U/25 μL , 0.25 U/25 μL , 0.1 U/25 μL , 0.05 U/25 μL , 0.025 U/25 μL , and 0.01 U/25 μL . Add 25 μL of each standard to triplicate wells.
5. Distribute 25 μL of 1X PARP Cocktail into each well using a multi-channel pipette.
6. The final reaction volume is 50 μL :

- i. PARP Inhibitor Study

| Reaction Component | Volume | Order of Addition |
|--|------------------|-------------------|
| Diluted test inhibitor or 1X PARP buffer | X μL | 1 |
| Diluted PARP-HSA Enzyme (0.5 unit) | Y μL | 2 |
| 1X PARP Cocktail | 25 μL | 3 |
| Total Volume | 50 μL | |
| Where X+Y= 25 μL | | |

Note: If X = 10 μL , make the concentration of your inhibitor 5-fold that of the final inhibitor concentration in the reaction since the reaction volume is 50 μL . In this example, Y = 15 μL . Dilute the PARP-HSA Enzyme to 0.5 U/15 μL in 1X PARP Buffer.

7. Incubate the strip wells at room temperature for 60 minutes.

DETECTION

1. Wash strip wells 2 times with 1X PBS + 0.1% Triton X-100 (200 μ L/well) followed by 2 washes with 1X PBS. Ensure that all the liquid is removed following each wash by tapping strip wells onto paper towels.
2. Add 50 μ L per well of Strep-HRP Working Solution. Incubate at room temperature for 60 minutes.
3. Wash strip wells 2 times with 1X PBS + 0.1% Triton X-100 (200 μ L/well) followed by 2 washes with 1X PBS. Ensure that all the liquid is removed following each wash by tapping strip wells onto paper towels.
4. Just before use mix equal volumes of PeroxyGlow™ A and B together and add 100 μ L per well. Immediately take chemiluminescent readings.

DATA INTERPRETATION

Typical chemiluminescent PARP standard curve and inhibition curves for the PARP inhibitors 3-aminobenzamide (provided in the kit), benzamide and 4- amino-1,8-naphthalimide are graphically represented below.

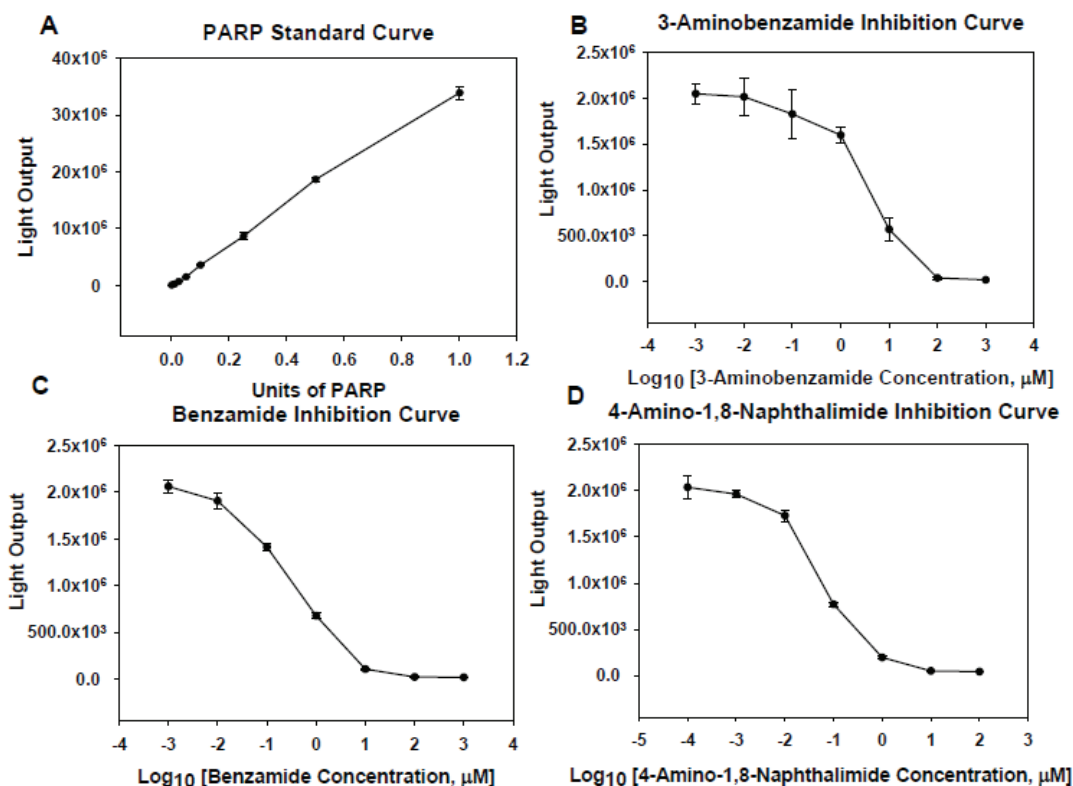


Figure 1. Graphical representation of the chemiluminescent readout of the PARP standard curve (Panel A) and inhibition curves for 3-aminobenzamide (Panel B), benzamide (Panel C), and 4-amino-1,4-naphthalimide (Panel D). Each point represents the median value from triplicates.

TROUBLESHOOTING

| Reaction Component | Volume | Order of Addition |
|---|---|---|
| No light output in wells with Inhibitor but present in wells with PARP alone. | PARP inhibitor is extremely potent. | Increase the serial dilutions of your inhibitor. |
| No light output in wells with PARP alone. | No light output in wells with no inhibitor, indicates PARP enzyme was not added to the wells. | Add 0.5 Units of PARP-HSA Enzyme to each well. |
| High background in wells with no PARP. | Poor washing. | Increase washes with 1X PBS + 0.1% Triton X-100 after the ribosylation reaction and incubation with Strep-HRP Working Solution. |

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