TREVIGEN® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

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

96 Tests

Cat# 4676-096-K

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

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Table of Contents

I. 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 by 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] Moreover, PARP inhibition promotes chemosensitization and radiosensitization of tumors.[12]

Trevigen's 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 the screening of PARP inhibitors and determining IC_{50} values. Important features of the assay include: 1) chemiluminescent, non-radioactive format; 2) higher throughput 96 test size; and 3) sensitivity down to 0.01 Units of PARP per well. Histone-coated 96-well white strip wells (Cat# 4678-096-P) are available separately for your convenience.

II. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The physical, chemical, and toxicological properties of the chemicals and reagents contained in the HT Universal Chemiluminescent PARP Assay Kit may not yet have been fully investigated. Therefore, Trevigen recommends the use of gloves, lab coats, and eye protection while using any of these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products. MSDS are available on request.

III. Materials Supplied

Catalog #	Component	Amount	Storage
4668-050-01	PARP-HSA, 10 U/µl	50 µl	-20°C
4671-096-02	*20X PARP Buffer (2 vials)	2 x 500 µl	-80°C
4671-096-03	*10X PARP Cocktail**	300 µl	-80°C
4678-096-P	*Histone-Coated White Strip Wells	96 wells	4°C
4667-50-03	*200 mM 3-Aminobenzamide	60 µl	-80°C
4671-096-04	10X Strep-Diluent	2 ml	4°C
4800-30-06	Strep-HRP	30 µl	4°C
4675-096-01	PeroxyGlow™ A	6 ml	4°C
4675-096-02	PeroxyGlow™ B	6 ml	4°C
4671-096-06	*10X Activated DNA	300 µl	-20°C

*Components marked with an asterisk can be stored at -20°C for one year in a manual defrost freezer.
**Contains biotinylated NAD.

IV. Materials/Equipment Required But Not Supplied

Reagents:

1. Inhibitors
2. PBS (4870-500)
3. PBS + 0.1% Triton X-100
4. Distilled water

Disposables:
1. 1 - 200 µl and 100-1000 µl pipette tips

Equipment:
1. Micropipettes
2. Multichannel pipettor 10 - 200 µl
3. Wash bottle or microstrip wells washer (optional)
4. 96-well plate reader

V. Reagent Preparation

1. 10X Strep-Diluent
This solution is used as a diluent for the Strep-HRP. Dilute **1:10** with dH_20 before use.

2. 20X PARP Buffer
Dilute the 20X PARP Buffer to **1X (1:20)** with dH_2O. The **1X** PARP Buffer is used to rehydrate the histone coated wells, and to dilute the enzyme, PARP Cocktail, and the inhibitors to be tested.

3. 10X PARP Cocktail
Dilute the 10X PARP Cocktail as follows:

10X PARP Cocktail (Cat# 4671-096-03)	2.5 µl/well
10X Activated DNA (Cat# 4671-096-06)	2.5 µl/well
1X PARP Buffer	20 µl/well

4. PARP Enzyme
The kit contains 50 µl of PARP-HSA enzyme at a concentration described in the enclosed Product Data Sheet. The enzyme should be diluted appropriately with **1X** PARP Buffer just before use. **Note: Diluted enzyme should be used immediately and any remainder discarded.**

5. PARP Inhibitors
The 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 µM to 10 mM. Serially dilute the stock 3-AB or your PARP inhibitor(s) with **1X** PARP Buffer and add to designated wells.

6. Strep-HRP
Just before use, dilute Strep-HRP (cat# 4800-30-06) 500-fold with **1X** Strep-Diluent (cat# 4671-096-04). A total of 50 µl/well of diluted Strep-HRP is required in the assay.

7. 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.

VI. PARP Inhibitor Assay Protocol

A. Ribosylation Reaction

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

1. Remove the 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 inhibitor of interest (prepared in section **V.5**) to appropriate wells.

3. Add diluted PARP enzyme (0.5 Unit/well prepared in Section **V.4**) to the wells containing inhibitor. Incubate for 10 minutes at room temperature.

4. Controls:
i. Negative Control: A negative control without PARP should be prepared to determine background absorbance.
ii. Activity Control for PARP Inhibitor Study: 0.5 unit/well PARP-HSA without inhibitors. These wells provide the 100% activity reference point.
iii. Optional PARP Standard Curve: Serially dilute the PARP-HSA standard in cold microtubes with **1X** PARP Buffer such that the total activity is 1 Unit/25 µl, 0.5 Units/25 µl, 0.25 Units/25 µl, 0.1 Units/25 µl, 0.05 Units/25 µl, 0.025 Units/25 µl, and 0.01 Units/25 µl. Add 25 µl of each standard to triplicate wells.

5. Distribute 25 µl of **1X** PARP Cocktail into each well using a multichannel pipettor.

6. The final reaction volume is 50 µl:
i. PARP Inhibitor Study

	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. Therefore, dilute the PARP-HSA enzyme to 0.5 units/15 µl in **1X** PARP Buffer.

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

B. Detection

1. Wash strip wells 2 times with **1X** PBS + 0.1% Triton X-100 (200 µl/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 µl per well of diluted Strep-HRP (prepared in section **V.6**). Incubate at room temperature for 60 minutes.
3. Wash strip wells 2 times with **1X** PBS + 0.1% Triton X-100 (200 µl/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 µl per well. Immediately take chemiluminescent readings.

VII. 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 (available from Trevigen) are graphically represented in Figure 1.

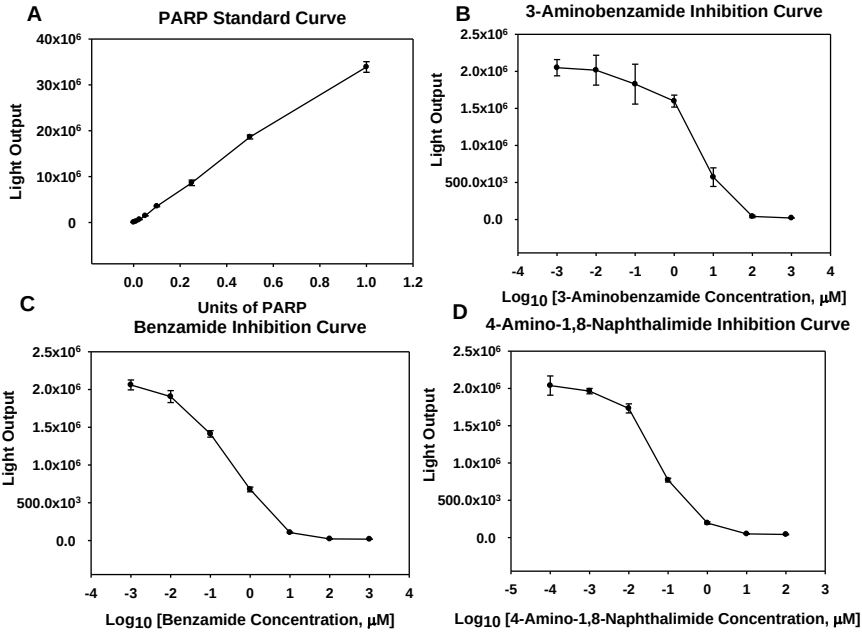

Figure 1. Graphical representation of the chemiluminescent readout of the PARP standard curve (Panel **A**) and inhibition curves for 3-aminobenzamide (Panel **B**), benzamide (Panel **C**), and 4-amino-1,4-naphthalimide (Panel **D**). Each point represents the median value from triplicates.

VIII. References

1. Satoh MS, Lindahl T. 1992. Role of poly(ADP-ribose) formation in DNA repair. *Nature* **356**: 356–8.
2. Golstein P, Kroemer G. 2007. Cell death by necrosis: Towards a molecular definition. *Trends Biochem Sci* **32**: 37-43.
3. D'Amours D, Sallmann FR, Dixit VM, Poirer GG. 2001. Gain-of-function of poly(ADP-ribose) polymerase-1 upon cleavage by apoptotic proteases: implications for apoptosis. *J Cell Science* **114**: 3771-78.
4. Eliasson M, Sampei K, Mandir AJ. 1997. Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. *Nat Med* **3**:1089- 95.
5. Miller MS, Zobre C, Lewis M. 1993. *In vitro* neuroprotective activity of inhibitors of poly-ADP ribose polymerase. *Soc Neurosci Abstr* **19**.1656
6. Pieper AA, Verma A, Zhang J, Snyder SH. 1999. Poly(ADP-ribose) polymerase, nitric oxide and cell death. *Trends Pharmacol Sci* **20**:171-81.
7. Thiemermann C, Bowes J, Myint FP, Vane JR. 1997. Inhibition of the activity of poly(ADP-ribose) synthase reduces ischemia-reperfusion injury in the heart and skeletal muscle. *Proc Natl Acad Sci USA* **94**:679-83.
8. Virag L, Szabo C. 2002. The therapeutic potential of Poly(ADPribose) Polymerase inhibitors. *Pharmacol Rev* **54**:375-429.
9. Tong WM, *et al*. 2002. Synergistic role of Ku80 and poly(ADP-ribose) Polymerase in sup-pressing chromosomal aberrations and liver cancer formation. *Cancer Res*. **62**:6990-6.
10. Kim MY, Mauro S, Gevry N, Lis JT, Kraus WL. 2004. NAD-Dependent Modulation of Chromatin Structure and Transcription by Nucleosome Binding Properties of PARP-1. *Cell* **119**:803–14.
11. Kauppinen TM, Swanson RA. 2005. Poly(ADP-ribose) polymerase-1 promotes microglial activation, proliferation, and matrix metalloproteinase-9-mediated neuron death. *J Immunol.* **174**:2288-96.
12. Curtin NJ. 2005. PARP inhibitors for cancer therapy. *Expert Rev Mol Med*. **7**:1-20.

IX. Troubleshooting

PROBLEM	CAUSE	SOLUTION
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 wasnot added to the wells.	Add 0.5 Unit of PARP to each well.
High background in wells with no PARP	Poor washing	Increase the number of washes with **1X** PBS + 0.1% Triton X-100 after the ribosylation reaction and incubation with Strep-HRP.

X. Related Products Available From Trevigen

Catalog #	Description	Size
4520-096-K	**PARP in vivo Pharmacodynamic Assay II**	**96 tests**
4677-096-K	**HT Universal Color PARP Assay Kit/w Histone Coated Strip Wells**	**96 samples**
4684-096-K	**HT Colorimetric PARP Apoptosis Assay**	**96 tests**
4685-096-K	**HT Chemiluminescent PARP Apoptosis Assay**	**96 tests**

Catalog #	Description	Size
4690-096-K	HT F Homogeneous PARP Inhibition Assay Kit	96 tests
4667-250-01	Recombinant Human PARP Enzyme	250 µl
4668-100-01	Recombinant Human PARP (High Specific Activity)	1000 Units
4682-096-K	HT Chemiluminescent PARG Assay Kit	96 tests
4683-096-K	HT Colorimetric PARG Assay Kit	96 tests
4667-50-11	Benzamide PARP inhibitor (8 mM)	100 µl
4667-50-10	6(5H)-Phenanthridinone PARP inhibitor (160 µM)	100 µl
4667-50-9	4-Amino-1,8-naphthalimide PARP inhibitor (800 µM)	100 µl

Accessories

Catalog #	Description	Size
4870-500-6	10X PBS, pH = 7.4	6 x 500 ml
4869-500-6	Apoptosis Grade™ H_2O	6 x 500 ml
4670-500-1	Biotinylated-NAD 250 µM	500 µl
4668-100-01	Recombinant Human PARP (HSA)	100 U
4667-50-06	Activated DNA	500 µl
4678-096-P	Histone-coated white strip well plate	1 plate

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

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