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**Universal Chemiluminescent
PARP Assay Kit With
Histone-Coated Strip wells**

**Chemiluminescent assay kit for
screening of PARP inhibitors, and
quantitation of PARP activity in cells.
Sufficient reagents for 96 strip wells.**

**Cat# 4676-096-K
Kit with Histone-Coated Strip wells**

Universal Chemiluminescent PARP Assay Kit 96 Tests

Cat# 4676-096-K: Kit with Histone-Coated Strip wells

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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 adjacent nuclear proteins. PARP may orchestrate the sequence of events that occurs in the DNA repair process^[1]. Induction of apoptosis occurs by depletion of the cellular NAD pool^[2]. Experimental models have shown that PARP inhibition prevents tissue damage in animal models of myocardial and neuronal ischemia, diabetes, septic shock, and vascular stroke^[3-10]. Recent data implicate a synergistic function of Ku80 and PARP-1 in minimizing chromosome aberrations and cancer development [8]. Trevigen's Universal 96-well PARP Assay Kit measures the incorporation of biotinylated Poly(ADP-ribose) onto histone proteins in a 96-well strip wells format. This assay is ideal for the screening of PARP inhibitors and for measuring the activity of PARP in cell extracts. Important features of the assay include: 1) chemiluminescent, non-radioactive format; 2) higher throughput 96 test size; and 3) sensitivity down to 0.0025 units of PARP per well. Trevigen offers two formats of the kit: Cat# 4675-096-K (Chemiluminescent Kit with histone reagent) and Cat# 4676-096-K (Chemiluminescent Kit with histone-coated strip wells). 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 Universal Colorimetric 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 Number	Component	Amount Provided	Temperature
4668-50-01	PARP-HSA	50 µl	-80°C
4671-096-02	*20X PARP Buffer	500 µl	-80°C
4671-096-03	*10X PARP Cocktail**	300 µl	-80°C
4678-096-P	*Histone-Coated White Strip wells	96	4°C
4667-50-03	*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	-80°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 or cells/tissue to be tested.
2. PBS + 0.1% Triton X-100
3. Distilled water
4. 0.2 N HCl or 5% Phosphoric acid
5. Phenylmethyl Sulfonyl Fluoride (PMSF) and other protease inhibitors
6. Triton X-100 or Nonidet P-40, and 1M NaCl for extract preparation.

Disposables:

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

Equipment:

1. Micropipettes
2. Multichannel pipettor 1 - 50 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** in **1X** PBS + 0.1% Triton X-100 before use.

2. 20X PARP Buffer

Dilute the 20X PARP Buffer to **1X (1:20)** with dH₂O. The **1X** PARP Buffer is used to dilute the enzyme, PARP Cocktail, the inhibitors to be tested (if required) and to prepare cell extracts.

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 Screening 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 serial dilutions of inhibitor of interest (prepared in section V.5) to appropriate wells.
2. 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.
3. Controls:
 - i. Activity control: 0.5 unit/ well PARP-HSA without inhibitors. These wells provide the 100% activity reference point.
 - ii. Negative control: A negative control without PARP should be prepared to determine background absorbance.
4. Distribute 25 µl of **1X** PARP Cocktail into each well using a multichannel pipettor.
5. The final reaction volume is 50 µl:

	<u>Volume</u>	<u>Order of Addition</u>
Diluted test inhibitor	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.

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

B. Detection

1. Wash strip wells 4 times with **1X** PBS + 0.1% Triton X-100 (200 μ l/well). 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 diluted Strep-HRP (prepared in section **V.6**). Incubate at room temperature for 60 minutes.
3. Wash strip wells 4 times with **1X** PBS + 0.1% Triton X-100 (200 μ l/well). 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.

VII. PARP Activity in Cell and Tissue Extracts

A. Processing Cells

1. **Non-adherent cells:** Centrifuge 2×10^6 to 1×10^7 non-adherent cells at 400 x g for 10 minutes at 4°C. Discard the supernatant. Suspend the cell pellet in 1 ml of ice-cold **1X** PBS and transfer to a prechilled 1.5 ml microtube. Centrifuge at 10,000 x g for 10 sec at 4°C. Discard the supernatant.

Adherent cells: Wash the adherent cells with **1X** PBS. Adherent cells may be harvested by scraping in 5 ml of ice-cold **1X** PBS or by gentle trypsinization. Transfer to a prechilled 15 ml tube. Centrifuge at 400 x g for 10 minutes at 4°C and discard the supernatant. Suspend the cell pellet in 1 ml of ice-cold **1X** PBS and transfer to a prechilled 1.5 ml microtube. Centrifuge at 10,000 x g for 10 sec at 4°C. Discard the supernatant.

B. Processing Tissue

1. Remove tissue and place in cold PBS in a 50 ml conical tube. Repeatedly wash the tissue with PBS to remove blood clots and other debris.
2. Transfer the tissue to a Petri dish on ice and mince the tissue to small pieces with surgical scissors.
3. Transfer the tissue pieces to a clean stainless steel sieve. Place the sieve with the tissue pieces in a Petri dish which contains about 20 ml of cold **1X** PBS.
4. Create a single cell suspension of the tissue as follows: Using a pestle or a round bottom tube, grind the tissue pieces thoroughly until the bulk of the tissue passes through the sieve.
5. Transfer the PBS containing the single cell suspension to a 50 ml conical tube. Fill with cold PBS and mix by inverting the tube several times. Let the tube stand on ice for 1 minute to allow large aggregates of tissue to settle out of solution.

6. Carefully transfer the supernatant containing the single cell suspension to a clean 50 ml conical centrifuge tube. Centrifuge at 400 x g for 10 minutes at 4°C. Discard the supernatant. Suspend the cell pellet in 1 ml of ice-cold **1X** PBS and transfer to a prechilled 1.5 ml microtube. Centrifuge at 10,000 x g for 12 sec at 4°C. Discard the supernatant.

C. Preparation of Extracts

1. Suspend the cell pellet obtained above in 5-10 pellet volumes of cold **1X** PARP Buffer containing 0.4 mM PMSF, other protease inhibitors, 0.4 M NaCl, and 1% Triton X-100 or 1% NP-40 nonionic detergent. Incubate the cell suspensions on ice, with periodic vortexing, for 30 minutes.
2. Microcentrifuge the disrupted cell suspension at 10,000 x g for 10 minutes at 4°C to remove insoluble material. Recover the supernatant to a fresh tube prechilled on ice. Occasionally, the pellet may float and can easily be removed with a pipet tip.
3. Determine the protein concentration of the cleared cell lysate. Use at least 20 μ g of protein per well in the assay.
4. Snap-freeze the cleared cell extract in small aliquots and store at -80°C. Avoid repeated freezing and thawing of the extract.

D. Ribosylation Reaction

Note: Do not premix the cell extract and the PARP Cocktail since PARP will autoribosylate in the presence of NAD.

1. Each sample will be in 3 wells. Add X μ l of **1X** PARP Buffer and Y μ l of your sample (containing at least 20 μ g of protein) into each of the designated 3 wells such that X + Y = 25 μ l.
2. Controls:
 - i. 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 μ l, 0.5 Units/25 μ l, 0.1 Units/25 μ l, 0.05 Units/25 μ l, and 0.01 Units/25 μ l, 0.005 Units/25 μ l, and 0.001 Units/25 μ l. Add 25 μ l of each standard to triplicate wells.
 - ii. Negative control: A negative control without PARP or cell extract should be included to provide the background absorbance that is subtracted from the experimental samples in the analysis of the data.
3. Distribute 25 μ l of **1X** PARP Cocktail into each well using a multichannel pipettor.
4. Incubate the strip wells at room temperature for 60 minutes. The incubation time can be extended if required.

5. The final reaction volume in each well is 50 μ l:

	Volume	Order of Addition
1X PARP Buffer	X μ l	1
Cell Extract or PARP Standard	Y μ l	2
1X PARP cocktail	25 μ l	3
Total volume	50 μ l	

Where X + Y = 25 μ l.

Notes: Y = 25 μ l for the PARP Standards and X = 0 μ l

X = 25 μ l for the background wells.

E. Detection

1. Follow **Section VI D** in the PARP Inhibitor Screening Assay Protocol.

VIII. Data Interpretation.

Typical chemiluminescent PARP standard curves 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.

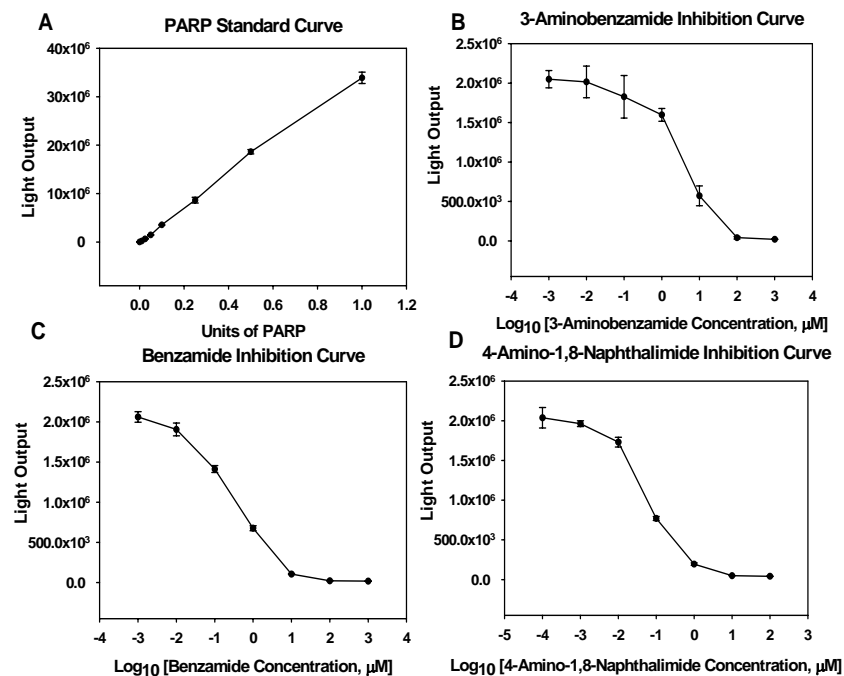


Figure 1. Graphical representation of the chemiluminescent readout of the PARP standard curve (Panel A) and inhibition curves for 3-aminobenzamide (Panel B), benzamide (Panel C), and 4-amino-1,4-naphthalimide (Panel D). Each point represents the median value from triplicates.

IX. References

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X. 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	If no color develops in the wells with no inhibitor, then PARP enzyme was not added to the wells.	Add 0.5 Unit of PARP to each well.
No light output in wells containing cell or tissue extracts	PARP expression in cells and tissues very low	Extend development time with TACS Sapphire to 1 hour.
		Add 1M NaCl to a final concentration of 0.4M in the cell extraction buffer
		Increase the volume and/or concentration of cell extract added to each well.
No light output in wells containing cell or tissue extracts	PARG activity in the extracts very high	Add ADP-HPD (Calbiochem, Cat.# 118415), a specific inhibitor of PARG, to a final concentration of 100 nM
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.

XI. Related Products Available From Trevigen

Catalog #	Description	Size
4870-500-6	10X PBS, pH 7.4	6 X 500 ml
4673-500-01	FITC-NAD	250 µl
4335-MC-100	Anti-poly(ADP-ribose), monoclonal	100 µg
4336-PC-100	Anti-poly(ADP-ribose), polyclonal	100 µl
4338-MC-50	Anti-poly(ADP-ribose) polymerase (PARP)	50 µg
4500-050-P	Poly(ADP-ribosylated) protein	50 µl
4668-100-01	Recombinant Human PARP Enzyme (HSA)	100 U
4668-500-01	Recombinant Human PARP Enzyme (HSA)	500 U
4668-2K-01	Recombinant Human PARP Enzyme (HSA)	2000 U
4667-50-06	Activated DNA	500 µl
4678-096-P	Histone-coated 96 well white strip wells	Each
4667-50-03	3-Aminobenzamide	100 µl
4667-50-09	4-Amino-1,8-Naphthalimide	100 µl
4667-50-10	6(5H)-Phenanthridinone	100 µl
4667-50-11	Benzamide	100 µl

XII. Appendix

Reagent composition:

- 1X PBS (pH 7.4):** 7.5 mM Na₂HPO₄, 2.5 mM NaH₂PO₄, 145 mM NaCl.
- Histone Solution:** 1 mg/ml Histones.
- 10X Strep Diluent:** Biotin-reduced proprietary blocking solution.
- 20X PARP Buffer:** Proprietary buffer solution.
- 10X PARP Cocktail:** Proprietary solution containing biotinylated NAD.
- PARP-HSA Enzyme:** PARP-HSA is provided at a concentration described in the enclosed Product Data Sheet.
- 3-Aminobenzamide:** 200 mM 3-aminobenzamide in Ethanol.
- PeroxyGlow™ A and PeroxyGlow™ B:** Chemiluminescent peroxidase substrates which, when mixed together, emit light in the presence of HRP.
- 10X Activated DNA:** Activated Herring Sperm DNA in 10 mM Tris-Cl (pH 8.0), 1 mM EDTA.
- Strep-HRP:** Provided at 500X Concentration

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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