DATA SHEET

HT Universal Colorimetric PARP Assay Kit with Histone-Coated Strip Wells 96 Tests

Cat# 4677-096-K

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

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 96-well strip well format. This assay is ideal for the screening of PARP inhibitors and determining IC50 values. Important features of the assay include: 1) colorimetric, nonradioactive format; 2) higher throughput 96 test size; and 3) sensitivity down to 0.01 Units of PARP per well. Histone-coated 96-well clear strip wells (4677-096- 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 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

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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
4677-096-P	*Histone-Coated Clear 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
4822-96-08	TACS-Sapphire™	10 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
2. PBS (cat# 4870-500)
3. PBS + 0.1% Triton X-100
4. Distilled water
5. 0.2N HCl or 5% Phosphoric acid

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 with 450 nm filter

V. Reagent Preparation

1. 10X Strep-Diluent
This solution is used as a diluent for the Strep-HRP. Dilute **1:10** with dH20 before use.

2. 20X PARP Buffer
Dilute the 20X PARP Buffer to **1X (1:20)** with dH2O. 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. TACS-Sapphire™
Prewarm TACS-Sapphire to room temperature before use. TACS-Sapphire is a colorimetric substrate that turns blue in the presence of Horseradish Peroxidase (HRP). The addition of an equal volume of 0.2 M HCl or 5% phosphoric acid stops the reaction to generate a yellow color stable for up to 60 minutes that can be read at 450 nm.

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 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-HAS 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.
ii. *Optional* PARP Standard Standard Volume Order of Addition Diluted PARP Standards 25 µl 1 **1X** PARP cocktail 25 µl 2 Total volume 50 µl
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. Add 50 µl per well of pre-warmed TACS-Sapphire™ colorimetric substrate and incubate, **in the dark**, for 15 minutes at room temperature. Stop the reactions by adding 50 µl per well of 0.2M HCl or 5% Phosphoric Acid and read the absorbance at 450 nm.

VII. Data Interpretation

Typical colorimetric 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.

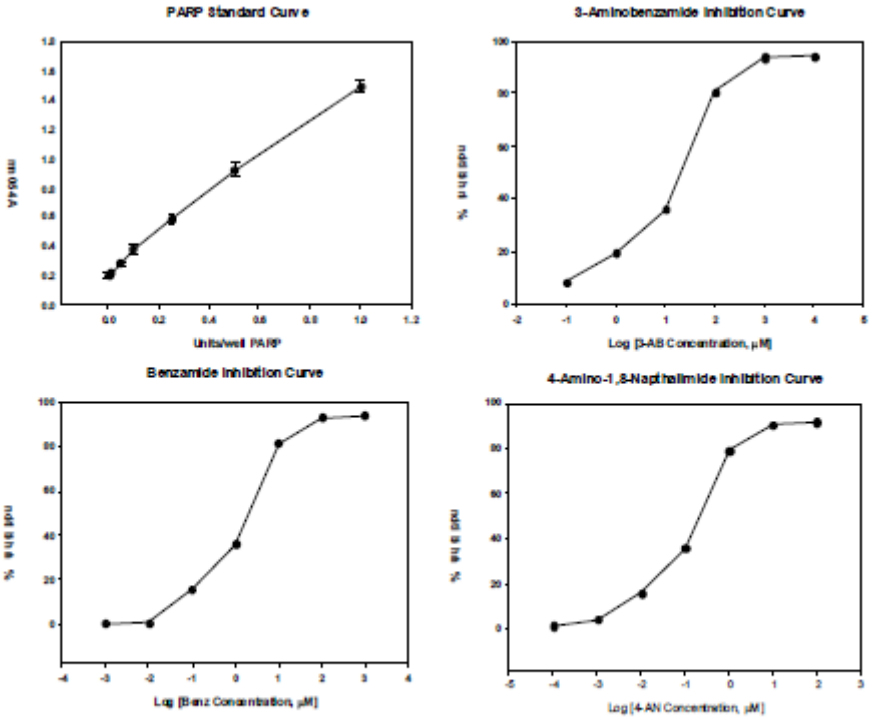

Figure 1. Graphical representation of the colorimetric readout of the PARP standard curve and inhibition curves for 3-aminobenzamide, benzamide, and 4-amino-1,4-naphthalimide. Each point represents the median value from triplicates.

VIII. References

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IX. Troubleshooting

PROBLEM	CAUSE	SOLUTION
No color in wells with Inhibitor but color is present in wells with PARP alone	PARP inhibitor is extremely potent	Increase the serial dilutions of your inhibitor
No color 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.
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.

X. Related Products Available From Trevigen

XI. Appendix

Reagent composition:

1. 1X PBS (pH 7.4): 7.5 mM Na2HPO4, 2.5 mM NaH2PO4, 145 mM NaCl.
2. 10X Strep Diluent: Biotin-reduced proprietary blocking solution.
3. 20X PARP Buffer: Proprietary buffer solution.
4. 10X PARP Cocktail: Proprietary solution containing biotinylated NAD.
5. PARP-HSA Enzyme: PARP-HSA is provided at a concentration described in the enclosed Product Data Sheet.
6. 3-Aminobenzamide: 200 mM 3-aminobenzamide in Ethanol.

7. TACS-Sapphire™ (cat# 4822-96-08): Peroxidase substrate readable at 630 nm (blue) or at 450 nm (yellow) after stopping reaction with 0.2 M HCl or 5% Phosphoric Acid.
8. 10X Activated DNA: Activated Herring Sperm DNA in 10 mM Tris-Cl (pH 8.0), 1 mM EDTA.
9. 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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