

TREVIGEN[®] Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

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 IC₅₀ values of PARP inhibitors.

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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 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, 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 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

| Catalog # | Component | Amount | Storage |
|-------------|-----------------------------------|-----------------|---------|
| 4668-050-01 | *PARP-HSA, 10 U / μ l | 50 μ l | -20 °C |
| 4671-096-02 | *20X PARP Buffer (2 vials) | 2 x 500 μ l | -80 °C |
| 4671-096-03 | *10X PARP Cocktail** | 300 μ l | -80 °C |
| 4677-096-P | *Histone-Coated Clear Strip wells | 96 wells | 4 °C |
| 4667-50-03 | *200 mM 3-Aminobenzamide | 60 μ l | -80 °C |
| 4671-096-04 | 10X Strep-Diluent | 2 ml | 4 °C |
| 4800-30-06 | Strep-HRP | 30 μ l | 4 °C |
| 4822-96-08 | TACS-Sapphire™ | 10 ml | 4 °C |
| 4671-096-06 | *10X Activated DNA | 300 μ l | -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 dH₂O 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-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:

| | <u>Volume</u> | <u>Order of Addition</u> |
|--|---------------|--------------------------|
| 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

| | <u>Volume</u> | <u>Order of Addition</u> |
|-------------------------|---------------|--------------------------|
| 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.

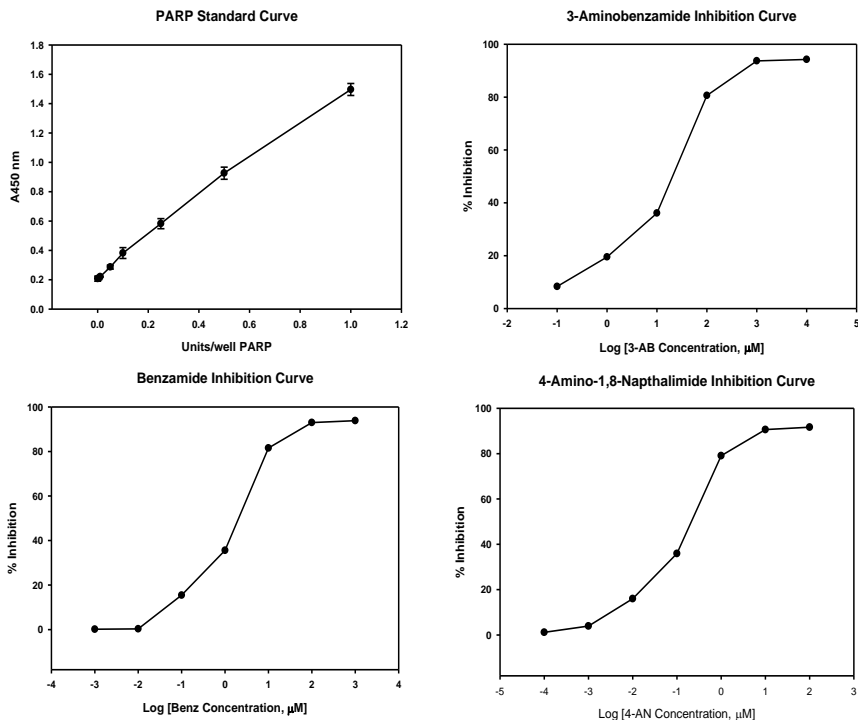


Figure 1. Graphical representation of the colorimetric readout of the PARP standard curve and inhibition curves for 3-aminobenzamide, benzamide, and 4-amino-1,4-naphthalimide. Each point represents the median value from triplicates.

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

| Catalog # | Description | Size |
|-------------------|--|-------------------|
| 4520-096-K | PARP in vivo Pharmacodynamic Assay II | 96 tests |
| 4676-096-K | HT Universal Chemiluminescent 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 |
| 4668-100-1 | 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 | 10X PBS, pH = 7.4 | 500 ml |
| 4869-500 | Apoptosis Grade™ H ₂ O | 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 |
| 4677-096-P | Histone-coated clear strip wells | 96 |

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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