PARP Universal Colorimetric Assay Kit

Catalog Number 4672-096-K

96-well colorimetric assay for screening Poly(ADP-ribose) Polymerase (PARP) inhibitors and quantitation of PARP activity in cells.

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

TABLE OF CONTENTS

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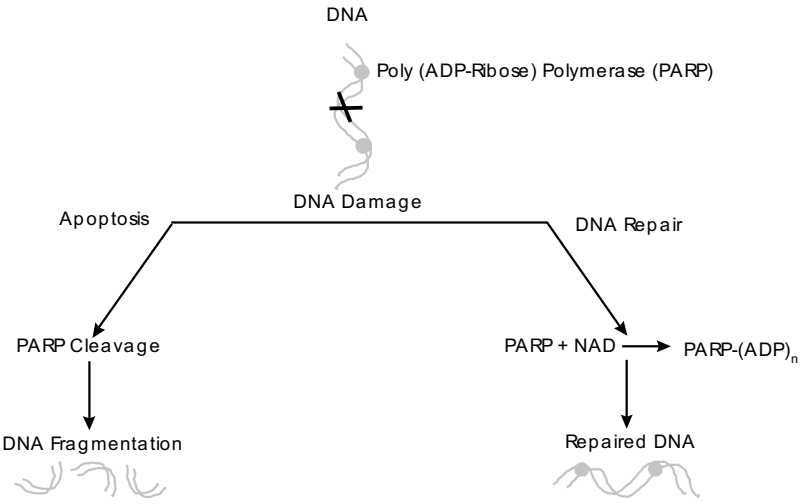
INTRODUCTION

Poly (ADP-ribosylation) of nuclear proteins is a post-translational event that occurs in response to DNA damage. Poly (ADP-ribose) Polymerase (PARP) is the enzyme catalyzing the NAD-dependent addition of ribose to adjacent nuclear proteins. PARP is an abundant nuclear protein present in all somatic cells. PARP plays an important role in DNA repair but can also lead to cell death by depleting the cellular NAD pool (1 - 2). PARP is activated when it becomes attached to regions of damaged DNA. The enzyme catalyzes its own ADP-ribosylation at glutamic acid residues and to a lesser extent nuclear proteins such as histones. Automodification of PARP aids in the release of PARP from DNA, permitting access by repair enzymes to the sites of DNA damage.

During apoptosis, PARP is specifically cleaved by members of the ICE family of proteases (*e.g.* Caspase-3). It is converted to an 85 kDa fragment with minimal activity that is not activated by damaged DNA. It apppears that PARP cleavage is a mechanism that prevents apoptotic cells from repairing their DNA. Experiments 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).



PRINCIPLE OF THE ASSAY

The PARP Universal Colorimetric Assay Kit measures the incorporation of biotinylated Poly (ADP-ribose) onto histone proteins in a 96-well plate. 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 colorimetric, non-radioactive format, higher throughput 96 test size, and sensitivity down to 0.01 units of PARP per well.

REAGENTS PROVIDED

Part Number	Component	Amount Provided	Storage
4668-050-01	PARP-HSA Enzyme	50 µL	≤ -80° C
4671-096-02	20X PARP Buffer*	500 µL	≤ -80° C
4671-096-03	10X PARP Cocktail*†	300 µL	≤ -80° C
4667-50-03	3-Aminobenzamide*	60 µL	≤ -80° C
4671-096-06	10X Activated DNA*	300 µL	≤ -80° C
4671-096-04	10X Strep-Diluent	2 mL	2 - 8° C
4822-96-08	TACS-Sapphire™	10 mL	2 - 8° C
4672-096-P	Histone-Coated Plate	1 plate	2 - 8° C
4800-30-06	Strep-HRP	30 µL	2 - 8° C

*These components can be stored at ≤ -20° C in a manual defrost freezer for up to 1 year, provided it is within the expiration date of the kit.

†Contains biotinylated NAD.

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

- Inhibitors or cells to be tested
- 1X PBS, pH 7.4 (without Ca^{2+} or Mg^{2+})
- Deionized or distilled water
- 0.2 M HCl or 5% phosphoric acid
- Phenylmethylsulfonyl fluoride (PMSF) and other protease inhibitors
- Triton X-100 or NP-40 and 1 M NaCl (for cell extract preparation)
- Detergent compatible protein determination reagents (*i.e.* Bradford or BCA)

Disposables

- Pipette tips (1 - 200 µL, 100 - 1000 µL)
- 1.5 mL microtubes
- 15 mL centrifuge tubes

Equipment

- Micropipettes
- Centrifuge
- Multi-channel pipette (1 - 50 µL)
- Microplate washer (optional)
- Ice bath
- 96 well plate reader (630 nm or 450 nm filter)

PRECAUTIONS

The physical, chemical, and toxicological properties of the chemicals and reagents in this kit may not yet have been fully investigated. The use of gloves, lab coats, and eye protection is recommended. MSDS are available upon request.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can alter the performance of the assay.

PREPARATION OF REAGENTS

Refer to the Appendix on page 9 for additional reagent composition information.

1. 1X Strep-Diluent

Dilute the 10X Strep-Diluent to 1X ten-fold (1:10) in 1X PBS (without Ca^{2+} or Mg^{2+}) before use. This solution is used as a diluent for the Strep-HRP.

2. 1X PARP Buffer

Dilute the 20X PARP Buffer to 1X twenty-fold (1:20) with deionized or distilled water. The 1X PARP Buffer is used to dilute the PARP-HSA Enzyme, PARP Cocktail, the inhibitors to be tested (if required), and to prepare cell extracts.

3. 1X PARP Cocktail

Dilute the 10X PARP Cocktail to 1X as follows.

10X PARP Cocktail	2.5 µL/well
10X Activated DNA	2.5 µL/well
1X PARP Buffer	20 µL/well

4. PARP-HSA Enzyme

This kit contains 50 µL of PARP-HSA Enzyme at a concentration described in the enclosed product insert. The enzyme should be diluted appropriately with 1X PARP Buffer just before use. **Note:** *Discard unused diluted enzyme.*

5. PARP Inhibitors

The 3-aminobenzamide (3-AB) is provided at 200 mM 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 and your PARP inhibitor(s) with 1X PARP Buffer and add to designated wells.

6. Strep-HRP

Just before use, dilute the Strep-HRP 500-fold with 1X Strep-Diluent. A total of 50 µL/well of diluted Strep-HRP is required in the assay.

PARP INHIBITOR SCREENING ASSAY PROTOCOL

A. Ribosylation Reaction

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

1. Add 25 µL of 1X PARP Cocktail to each well.
2. Controls:
   Activity Control - 1 unit/well of PARP-HSA Enzyme without inhibitors. These wells provide the 100% activity reference point.

   Negative Control - A negative control without PARP should be prepared to determine background absorbance.
3. Add inhibitor(s) of interest (prepared on page 4, step 5).
4. Add 1 unit/well of PARP-HSA Enzyme (prepared on page 4, step 4).

   The final reaction volume is 50 µL.

	Volume	Order of Addition
1X PARP Cocktail	25 µL	1
Diluted test inhibitor	X µL	2
Diluted PARP-HSA Enzyme (1 unit)	Y µL	3
Total volume	50 µL	

Where X + Y = 25 µL
Example: If X = 10 µL, make the working concentration of your inhibitor 5-fold that of the final concentration in the reaction. In this example, Y = 15 µL. Therefore, dilute the PARP-HSA Enzyme to 1 unit/15 µL.

5. Incubate the plate at room temperature for 60 minutes. The extent of ribosylation is time dependent, therefore, this step can be extended if desired.

B. Detection

1. Wash the plate 4 times with 1X PBS (200 µL/well). Ensure that all the liquid is removed following each wash by inverting the plate and blotting it against clean paper towels.
2. Add 50 µL of diluted Strep-HRP to each well. Incubate for 20 minutes at room temperature.
3. Wash the plate 4 times with 1X PBS (200 µL/well). Ensure that all the liquid is removed following each wash by inverting the plate and blotting it against clean paper towels.
4. Add 50 µL of TACS-Sapphire to each well and incubate for 10 - 30 minutes **in the dark.** TACS-Sapphire is a horseradish-peroxidase (HRP) substrate generating a soluble blue color. Color development should be monitored and the plate read at 630 nm. The reaction can be stopped by adding 50 µL of 0.2 M HCl or 5% phosphoric acid per well and the plate can then be read at 450 nm.

PARP ACTIVITY IN CELL EXTRACTS

A. Preparation of Cell Extracts

1. **Non-Adherent Cells** - Centrifuge 2 x 10^6 to 1 x 10^7 non-adherent cells at 400 x g for 10 minutes at 2 - 8° C. Discard the supernate. 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 seconds at 2 - 8° C. Discard the supernate. Suspend the cell pellet 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 non-ionic detergent.

   Adherent Cells - Wash the adherent cells with 1X PBS. Cells in monolayer may be harvested by scraping in 5 mL of ice cold 1X PBS or gentle trypsinization. Transfer to a pre-chilled 15 mL tube. Centrifuge at 200 x g for 10 minutes at 2 - 8° C. Discard the supernate. Resuspend the cell pellet in 1 mL of ice cold 1X PBS and transfer to a pre-chilled 1.5 mL microtube. Centrifuge at 10,000 x g for 12 seconds at 2 - 8° C. Discard the supernate. Resuspend the cell pellet 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 non-ionic detergent.
2. Incubate the cell suspension on ice with periodic vortexing for 30 minutes.
3. Microcentrifuge the disrupted cell suspension at 10,000 x g for 10 - 20 minutes at 2 - 8° C to remove insoluble material. Transfer the supernate to a fresh tube pre-chilled on ice. If the supernate is cloudy, repeat the centrifugation.
4. Determine the protein concentration of the clear supernate using a Bradford or similar assay that is compatible with detergents. Use at least 20 μg of protein per well in the PARP assay.
5. If samples are not used immediately, snap-freeze the clear cell extract supernates in small aliquots and store at ≤ -80° C. Avoid repeated freezing and thawing.

B. Ribosylation Reaction

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

1. Add 25 µL of 1X PARP Cocktail to each well.
2. Each sample will be in 3 wells. Add Y µL of your sample (containing at least 20 µg of protein) into each of the designated 3 wells.
3. Controls:
   PARP Standard Curve - Serially dilute the PARP-HSA Enzyme in cold microtubes with 1X PARP Buffer such that the total activity is 1 unit/25 µL, 0.5 units/25 µL, 0.1 units/25 µL, 0.05 units/25 µL, and 0.01 units/25 µL. Add 25 µL of each standard to triplicate wells.

   Negative Control - Use 25 µL of 1X PARP Buffer as a negative control without PARP-HSA Enzyme or cell extract. This is included to provide the background absorbance that is subtracted from the experimental samples in the analysis of the data.

   The final reaction volume in each well is 50 µL.

	Volume	Order of Addition
1X PARP Cocktail	25 µL	1
1X PARP Buffer	X µL	2
Cell Extract or PARP Standard	Y µL	3
Total Volume	50 µL	

X + Y = 25 µL Y = 25 µL for the PARP Standards and X = 0 µL
X = 25 µL for the Negative Control and Y = 0 µL.

4. Incubate the plate at room temperature for 60 minutes. The extent of ribosylation is time dependent; therefore, this step can be extended if desired.

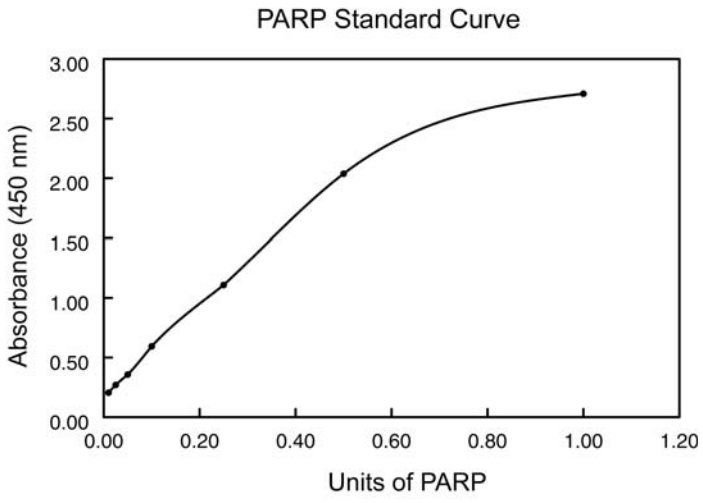
C. Detection

1. Wash the plate 4 times with 1X PBS (200 µL/well). Ensure that all the liquid is removed following each wash by inverting the plate and blotting it against clean paper towels.
2. Add 50 µL of diluted Strep-HRP to each well. Incubate for 20 minutes at room temperature.
3. Wash the plate 4 times with 1X PBS (200 µL/well). Ensure that all the liquid is removed following each wash by inverting the plate and blotting it against clean paper towels.
4. Add 50 µL of TACS-Sapphire to each well and incubate for 20 - 30 minutes **in the dark.** TACS-Sapphire is an HRP substrate generating a soluble blue color. Color development should be monitored and the plate read at 630 nm. The reaction can be stopped by adding 50 µL of 0.2 M HCl or 5% phosphoric acid per well and the plate can then be read at 450 nm.

DATA INTERPRETATION

These curves are for demonstration only. Curves should be generated for each set of samples assayed.

A.



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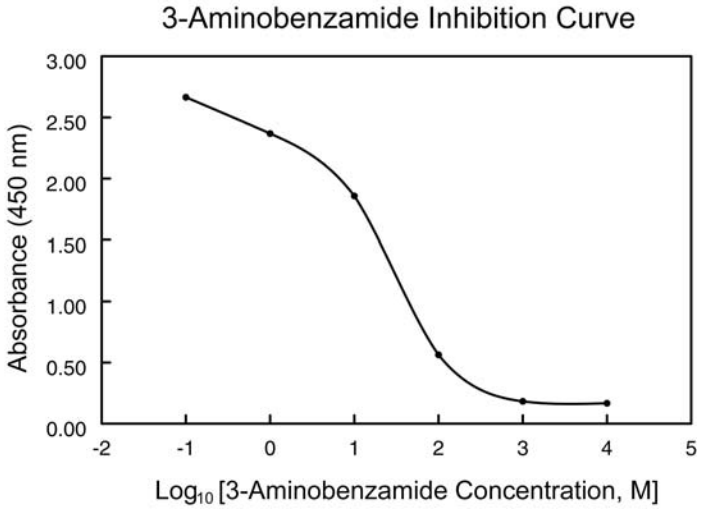

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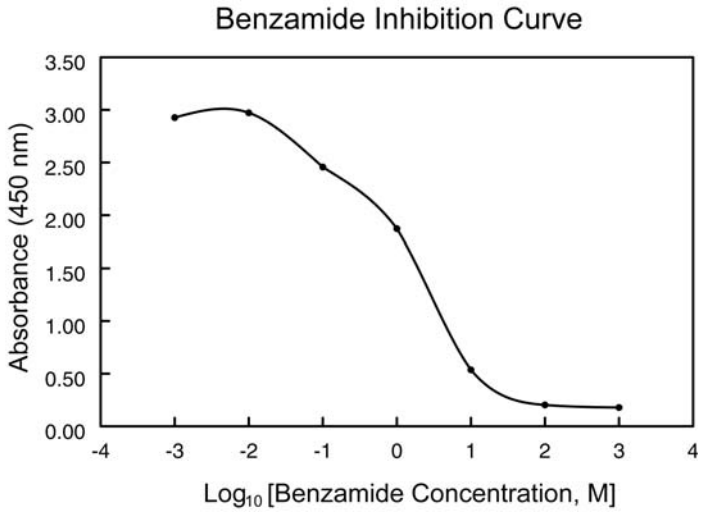

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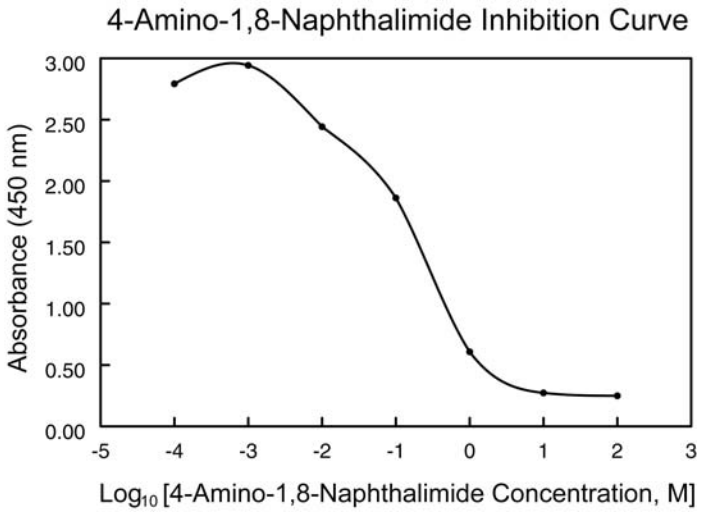

Figure 1: Each point represents the median value from triplicates.

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
No color in wells with inhibitor but color is present in wells with PARP alone	PARP inhibitor is extremely potent	Increase serial dilutions of your inhibitor
No color in Activity Control wells	If no color develops in the wells with no inhibitor, the PARP-HSA Enzyme was not added to the wells	Add 1 unit of PARP-HSA Enzyme to each well
No color development in wells containing cell extracts	PARP expression in cells and tissues very low	Extend development time with TACS-Sapphire to 1 hour Add 1 M NaCl to a final concentration of 0.4 M in the cell extraction buffer Increase the volume and/or concentration of cell extract added to each well
High background in wells with no PARP	Poor washing	Increase the number of washes with 1X PBS after the ribosylation reaction and incubation with Strep-HRP

APPENDIX

Reagent Composition

1X PBS (pH 7.4)
7.5 mM disodium hydrogen phosphate (Na_2HPO_4), 2.5 mM sodium dihydrogen phosphate (NaH_2PO_4), 145 mM sodium chloride (NaCl)

10X Strep Diluent
Biotin-reduced proprietary blocking solution

20X PARP Buffer
Proprietary blocking 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

TACS-Sapphire
Peroxidase substrate readable at 630 nm (blue) or at 450 nm (yellow) after stopping the reaction with 0.2 M HCl or 5% phosphoric acid

10X Activated DNA
Activated Herring Sperm DNA in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA

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NOTES