TREVIGEN® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

HT Chemiluminescent PARG Assay Kit

Screening assay for inhibitors and relative quantitation of activity. Sufficient reagents for 96 strip wells.

Cat# 4682-096-K

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Table of Contents

I. Introduction

Poly(ADP-ribose) glycohydrolase (PARG) degrades poly(ADP-ribose) (PAR) polymers synthesized by poly(ADP-ribose) polymerase (PARP1). When activated by DNA strand breaks, PARP1 uses NAD as a substrate to form ADP-ribose polymers on itself and on specific acceptor proteins such as histones, DNA polymerases, DNA ligases, p53, and Fos. These polymers are in turn rapidly degraded by PARG, a ubiquitously expressed exo- and endoglycohydrolase. Excessive activation of PARP1 leads to NAD depletion and cell death during ischemia and other conditions that generate extensive DNA damage. PARG may maintain the active state of PARP1 by continuously removing inhibitory ADP-ribose residues from PARP1.[1] The regulation of PARG activity may therefore, influence PARP-mediated cell death. First, PARG inhibition could slow the turnover of PAR and thereby limit NAD depletion. Second, PARG inhibition could prevent the removal of PAR from PARP1. Because PARP1 is inhibited by extensive poly-(ADP-ribosyl)ation, PARG inhibitors could thereby indirectly inhibit PARP1 activity.[2-10] Prior work has shown that the PARG inhibitor gallotannin can markedly reduce death of astrocytes after oxidative stress.[11] Trevigen's HT Chemiluminescent PARG Assay Kit measures the loss of biotinylated PAR from histones attached to strip wells in a 96 well format and is ideal for the screening of PARG inhibitors and for measuring the relative activity of PARG 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 250 pg of PARG per well. Trevigen offers histone-coated 96 white strip well plates (Cat# 4678-096-P) and other components of the PARG assay 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 Chemiluminescent PARG 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

Cat#	Component	Amount	Storage
4680-096-01	PARG, 1 µg/ml	300 µl	−80 °C
4680-096-02	10X PARG Buffer	8 ml	4 °C
4680-096-03	DEA, 100 mM	200 µl	−80 °C
4668-050-01	PARP-HSA, 10 units/µL	50 µL	−20 °C
4671-096-02	20X PARP Buffer	500 µL	−80 °C
4671-096-03	10X PARP Cocktail**	300 µL	−80 °C
4671-096-06	10X Activated DNA	300 µL	−80 °C
4678-096-P	Histone-Coated Strip Wells	96 wells	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

*****Components stored at −80 °C are stable at −20 °C for one year in a manual defrost freezer. Store PARG and PARP-HSA at −20 °C to avoid freeze-thaw of enzymes.

**Contains biotinylated NAD.

IV. Materials/Equipment Required But Not Supplied

Reagents:

1. Inhibitors or cells/tissue to be tested.
2. PBS
3. PBS + 0.1% Triton® X-100
4. Distilled water
5. Phenylmethyl Sulfonyl Fluoride (PMSF) and other protease inhibitors
6. Triton® X-100 or Nonidet P-40 for extract preparation

Disposables:

1. 1–200 µl and 100–1000 µl pipette tips
2. Eppendorf tubes

Equipment:

1. Micropipettes
2. Multichannel pipettor 1–50 µl
3. Wash bottles or microstrip wells washer (optional)
4. 96-well strip wells reader for chemiluminescence

V. Reagent Preparation

1. PBS Wash Solution
   Prepare 500 ml of 1X PBS in a wash bottle for washing the strip wells.

2. PBS-Triton® X-100 Wash Solution
   Prepare 500 ml of 1X PBS solution containing 0.1% (v/v) Triton® X-100 in a wash bottle for washing the strip wells.

3. Strep-HRP
   Just before use, dilute Strep-HRP (Cat# 4800-30-06) 500-fold with 1X PARG buffer. A total of 50 µl/well of diluted Strep-HRP is required in the assay.

4. 1X PARP Buffer
   Dilute 300 µl of the 20X PARP buffer (Cat# 4671-096-02) to 1X (1:20) with 5.7 ml of dH_2O. The 1X PARP buffer is used to dilute the PARP enzyme and to prepare the 1X PARP cocktail.

5. 1X PARP Cocktail
   Prepare the 1X 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

6. PARP Enzyme
   The kit contains 50 µl of PARP-HSA enzyme at a concentration of 10 Units/µl. The enzyme should be diluted with 1X PARP buffer to 0.04 Units/µl just before use. For example, if you intend to ribosylate the whole plate at once, dilute 12 µl of the PARP enzyme with 2988 µl of 1X PARP buffer and use 25 µl per well. ***Note:*** Diluted enzyme should be mixed by inversion and used immediately and any remainder discarded.

7. 1X PARG Buffer
   Dilute 8 ml of the 10X PARG buffer (Cat# 4680-096-02) to 1X (1:10) with dH_2O. The 1X PARG buffer is used to dilute the PARG enzyme, the Strep-HRP, the inhibitors to be tested (if required), and to prepare cell extracts.

8. PARG Enzyme
   a. Standard Curve (for columns 1 to 3, rows C-H, Fig. 3)
   Serially dilute the 1 µg/ml PARG standard (Cat# 4680-096-01) in microtubes with 1X PARG buffer to make at least 200 µl of each dilution. The recommended final concentrations are 100 ng/ml, 50 ng/ml, 25 ng/ml, 10 ng/ml, 5 ng/ml, and 1 ng/ml. The standard curve requires 50 µl/well of each PARG dilution and each is performed in triplicate. ***Note:*** Diluted enzyme should be should be mixed by inversion and used immediately and any remainder discarded. The following table describes a serial dilution protocol for PARG Standard Curve:

Final [PARG]	**100 ng/ml → 50 ng/ml → 25 ng/ml → 10 ng/ml → 5 ng/ml → 1 ng/ml**					
[PARG]	40 µl [1µg/ml]	200 µl [100ng/ml]	200 µl [50ng/ml]	200 µl [25ng/ml]	200 µl [10ng/ml]	200 µl [5ng/ml]
1X PARG Buffer	360 µl	200 µl	200 µl	300 µl	200 µl	800 µl

   b. PARG Inhibitor Screen (for example, columns 4 to 6, rows A-H, Fig. 3)
   Determine number of wells needed for Inhibitor screen. Each inhibitor well test requires 25 µl of 100 ng/ml PARG. Dilute enzyme stock 1:10 in 1X PARG Buffer. Mix diluted enzyme by inversion and use immediately. Discard any remainder. For example, the PARG inhibitor DEA titration (columns 4 to 6, rows A-H, Fig.3) minimally requires 600 µl of 100 ng/ml PARG (60 µl of 1 µg/ml PARG plus 540 µl 1X PARG Buffer).

9. PARG Inhibitors
   DEA (6,9-diamino-2-ethoxyacridine lactate monohydrate) is provided at 100 mM in DMSO as a control PARG inhibitor. DEA will inhibit the activity of PARG at a wide range of concentrations from 1 µM to 1 mM. In Eppendorf tubes, serially dilute the stock DEA or your PARG inhibitor(s) with 1X PARG buffer to twice their final concentrations. The following table describes a serial dilution protocol for DEA:

Final [DEA]	**2 mM → 200 µM →100 µM → 40 µM → 20 µM → 2 µM → 0.2 µM**						
[DEA]	10 µl [100 mM]	50 µl [2 mM]	250 µl [200 µM]	200 µl [100 µM]	250 µl [40 µM]	50 µl [20 µM]	50 µl [2 µM]
1X PARG Buffer	490 µl	450 µl	250 µl	300 µl	250 µl	450 µl	450 µl

10. Cell Extraction Buffer
    Prepare 10 ml of the following cell extraction buffer and store at 4 °C:

10X PARG buffer (Cat# 4680-096-02)	1 ml
5 M NaCl	800 µl
20% Triton® X-100 or 20% NP40	450 µl
200 mM PMSF	20 µl
dH_2O	7.73 ml

11. PeroxyGlow™ A and B Chemiluminescent Substrates
    Bring PeroxyGlow A and B to room temperature. 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 plate reader for luminescence. A total of 100 µl/well is required in the assay.

VI. PARG Inhibitor Screening Assay Protocol

Trevigen's HT Chemiluminescent PARG Assay Kit measures the loss of biotinylated PAR from histones attached to strip wells in a 96 well format. The histones attached to the plate wells are first poly(ADP) ribosylated by PARP using a biotinylated NAD substrate. The biotinylated PAR attached to the histones is subsequently hydrolyzed by the action of PARG in the standard or in the experimental samples. Any remaining biotinylated PAR is measured by incubation with Streptavidin-HRP and a chemiluminescent substrate for HRP. The extent of hydrolysis is reflected by the loss in light output compared to that obtained in the absence of PARG. Inhibitors of PARG reduce the hydrolysis of the biotinylated PAR on the histones, thus minimizing the loss in lightoutput compared to PARG alone.

When performing the PARG inhibitor protocol, the assay should be performed in triplicate and include the PARG standard curve. A general plate setup includes a negative PARP control, a negative PARG control, a PARG standard curve, serial dilutions of DEA PARG inhibitor (optional), and serial dilutions of inhibitor to be tested. A suggested plate setup is shown in Figure 3. The wells devoted to the cell extracts would be omitted in this case.

A. Ribosylation Reaction

Note: Do not premix the PARP-HSA enzyme and the 1X PARP cocktail since PARP will autoribosylate in the presence of NAD.

1. During this step PARP-HSA ribosylates the histones on the histone-coated strip wells. Remove the histone-coated strip wells from the wrapper and select the number of strip wells that you need.

2. Add 25 µl of diluted PARP enzyme (0.04 Units/µl prepared in Section V.6) to all the wells except that for the PARP negative control (wells A1 to A3, Fig.3). Add 25 µl of 1X PARP Buffer to wells A1 to A3.

3. Distribute 25 µl of 1X PARP cocktail (prepared as described in Section V.5) into each well using a multichannel pipettor.

   Note: The final reaction volume for the ribosylation reaction is 50 µl with 1 unit of PARP.

4. Cover and incubate the strip wells at room temperature for 30 min.

5. Wash strip wells 2 times with 1X PBS + 0.1% Triton® X-100 (200 µl/well) and 2 times with 1X PBS. It is important to remove all liquid and bubbles before performing the hydrolysis reaction (VI.B) to reduce well to well variability. Ensure that all the liquid is removed following each wash by tapping strip wells onto paper towels.

B. Hydrolysis Reaction

1. Prepare negative control and standard curve samples:

   a. *PARP Negative control:* Add 50 µl of 1X PARG Buffer (wells A1 to A3, Fig. 3). This control measures the nonspecific binding of Strep-HRP to the histones in the strip wells.

   b. *PARG Negative control:* Add 50 µl of 1X PARG Buffer (wells B1 to B3, Fig. 3). This control (without PARG) provides the 100% reference light output.

   c. *PARG Standard Curve:* Serially dilute the 1 µg/ml PARG standard in microtubes with 1X PARG buffer as described in Section V.8 above. Add 50 µl of each PARG dilution to triplicate wells (columns 1 to 3, rows C to H, Fig. 3).

2. Prepare inhibitor samples ***(must add inhibitor to plate before PARG)***:
   a. In 1.5 ml tubes, prepare twice-concentrated serial dilutions of the control DEA PARG inhibitor and/or your inhibitor in 1X PARG buffer as described in Section V.9 above.

   b. Transfer 25 µl of each 2X inhibitor concentration to triplicate wells (columns 4 to 6, rows A to G).

   c. To wells H4 to H6 add 25 µl 1X PARG Buffer.

   d. Transfer 25 µl of 100 ng/µl PARG to inhibitor wells (columns 4 to 6, rows A to H) and mix upon addition.

   Note: The final concentration of PARG will be 50 ng/ml and the inhibitors will be at their final 1X concentrations in a 50 µl reaction volume.

3. Cover and incubate the strip wells at room temperature for 30 min.

C. Detection

1. Wash strip wells 2 times with 1X PBS + 0.1% Triton® X-100 (200 µl/well) and 2 times with 1X PBS. It is important to remove all liquid and bubbles before performing the detection reaction to reduce well to well variability. 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.3). Cover and incubate at room temperature for 30 min.

3. Wash strip wells 2 times with 1X PBS + 0.1% Triton® X-100 (200 µl/well) and 2 times with 1X PBS. Ensure that all the liquid is removed following each wash by tapping strip wells onto paper towels.

4. Warm PeroxyGlow A and B to room temperature. Just before use mix equal volumes of PeroxyGlow™ A and B together and add 100 µl per well. Immediately take chemiluminescent readings.

VII. PARG Activity in Cell and Tissue Extracts

When performing the PARG activity protocol, the assay should be performed in triplicate. A general plate setup includes a negative PARP control, a negative PARG control, a PARG standard curve, and serial dilutions of extract to be tested. A general plate setup is shown in Figure 3 for testing of four extracts. The wells devoted to the PARG inhibitors would be omitted in this case.

A. Processing Cells

Suspension cells: Centrifuge 2 x 10^6 to 1 x 10^7 suspension cells at 400 x g for 10 min 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 and proceed to step VII.C.

Adherent cells: Wash 2 x 10^6 to 1 x 10^7 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 min 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 and proceed to step VII.C.

B. Processing Tissue

1. Remove tissue and place in cold PBS in a 50 ml conical tube. Repeatedly wash the tissue with cold 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 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 min 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. Count and record the number of cells. Centrifuge at 400 x g for 10 min at 4 ºC. 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 and proceed to step VII.C.

C. Preparation of Extracts

1. Suspend the cell pellet in 5–10 pellet volumes of cold cell extraction buffer from Step V.9. Incubate the cell suspensions on ice, with periodic vortexing, for 30 min.

2. Microcentrifuge the disrupted cell suspension at 10,000 x g for 10 min at 4ºC 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 10 µg of protein per well in the assay. ***Note:*** 1X PARG buffer contains 0.1 mg/ml BSA.

4. Snap-freeze the cleared cell extract in small aliquots and store at −80 ºC. Avoid repeated freezing and thawing of the extract.

D. Ribosylation Reaction

Note: Do not premix the cell extract and the 1X PARP cocktail since PARP will autoribosylate in the presence of NAD.

1. Perform the ribosylation reaction as described in Section VI A.

E. Hydrolysis Reaction

1. Each sample will be in triplicate. Distribute 50 µl of your sample into each of the designated 3 wells. ***Note:*** serial dilutions of your sample in 1X PARG assay buffer may be required to optimize PARG activity determination (see Fig. 3).

2. Controls:

   a*. PARP Negative control:* Add 50 µl of 1X PARG Buffer (wells A1 to A3, Fig. 3). This control measures the nonspecific binding of Strep-HRP to the histones in the strip wells.

   b. *PARG Negative control:* Add 50 µl of 1X PARG Buffer (wells B1 to B3, Fig. 3). This control (without PARG) provides the 100% reference light output.

   c. *PARG Standard Curve:* Serially dilute the 1 µg/ml PARG standard in microtubes with 1X PARG buffer as described in Section V.8 above. Add 50 µl of each PARG dilution to triplicate wells (columns 1 to 3, rows C to H, Fig. 3).

3. Incubate the strip wells at room temperature for 30 min.

F. Detection

1. Follow Section VI. C in the PARG Inhibitor Screening Assay Protocol.

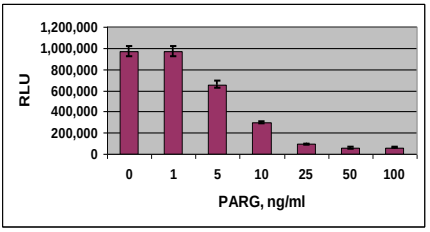
VIII. Data Interpretation

1. Calculate the average light output value and standard deviations of all triplicates and subtract the value of "No PARP Control" (A1 to A3) to obtain relative light unit values (RLU).

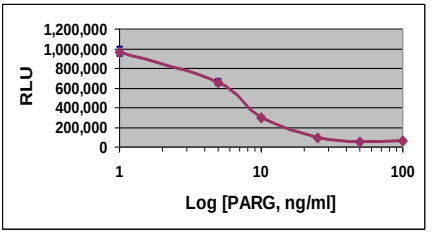
2. In EXCEL, use column chart plot or scatter plot to illustrate PARG Activity (Figure 1, A and B). PARG Standard values are generated using columns 1-3, rows B-H. The concentration of the PARG standards (X axis) are plotted against their relative light unit values (Y axis). When using a scatter plot, a logarithmic scale is used on the X axis.

Figure 1: PARG Standards

A. Chart Plot

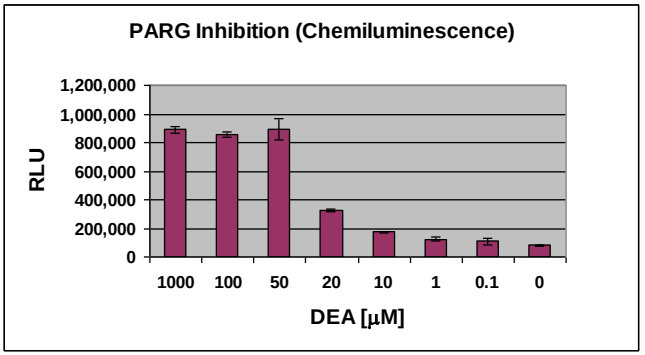


B. Scatter Plot



3. In EXCEL, use column chart plot to illustrate PARG inhibition by inhibitor (Figure 2). The inhibition of PARG caused by your inhibitor will be reflected as a two fold *increase* in the observed light output value relative to that caused by 50 ng/ml PARG in the absence of inhibitor. The inhibitor concentrations (X axis) are plotted against their relative light unit values (Y axis).

Figure 2: Chart Plot of Inhibitor Titration



	1	2	3	4	5	6	7	8	9	10	11	12
A	No PARP	No PARP	No PARP	1 mM DEA + 50 ng/ml PARG	1 mM DEA + 50 ng/ml PARG	1 mM DEA + 50 ng/ml PARG	Cell/ Tissue Extract #1 20 µg Protein	Cell/ Tissue Extract #1 20 µg Protein	Cell/ Tissue Extract #1 20 µg Protein	Cell/ Tissue Extract #3 20 µg Protein	Cell/ Tissue Extract #3 20 µg Protein	Cell/ Tissue Extract #3 20 µg Protein
B	No PARG	No PARG	No PARG	100 µM DEA + 50 ng/ml PARG	100 µM DEA + 50 ng/ml PARG	100 µM DEA + 50 ng/ml PARG	Cell/ Tissue Extract #1 10 µg Protein	Cell/ Tissue Extract #1 10 µg Protein	Cell/ Tissue Extract #1 10 µg Protein	Cell/ Tissue Extract #3 10 µg Protein	Cell/ Tissue Extract #3 10 µg Protein	Cell/ Tissue Extract #3 10 µg Protein
C	PARG 1 ng/ml	PARG 1 ng/ml	PARG 1 ng/ml	50 µM DEA + 50 ng/ml PARG	50 µM DEA + 50 ng/ml PARG	50 µM DEA + 50 ng/ml PARG	Cell/ Tissue Extract #1 5 µg Protein	Cell/ Tissue Extract #1 5 µg Protein	Cell/ Tissue Extract #1 5 µg Protein	Cell/ Tissue Extract #3 5 µg Protein	Cell/ Tissue Extract #3 5 µg Protein	Cell/ Tissue Extract #3 5 µg Protein
D	PARG 5 ng/ml	PARG 5 ng/ml	PARG 5 ng/ml	20 µM DEA + 50 ng/ml PARG	20 µM DEA + 50 ng/ml PARG	20 µM DEA + 50 ng/ml PARG	Cell/ Tissue Extract #1 2 µg Protein	Cell/ Tissue Extract #1 2 µg Protein	Cell/ Tissue Extract #1 2 µg Protein	Cell/ Tissue Extract #3 2 µg Protein	Cell/ Tissue Extract #3 2 µg Protein	Cell/ Tissue Extract #3 2 µg Protein
E	PARG 10 ng/ml	PARG 10 ng/ml	PARG 10 ng/ml	10 µM DEA + 50 ng/ml PARG	10 µM DEA + 50 ng/ml PARG	10 µM DEA + 50 ng/ml PARG	Cell/ Tissue Extract #2 20 µg Protein	Cell/ Tissue Extract #2 20 µg Protein	Cell/ Tissue Extract #2 20 µg Protein	Cell/ Tissue Extract #4 20 µg Protein	Cell/ Tissue Extract #4 20 µg Protein	Cell/ Tissue Extract #4 20 µg Protein
F	PARG 25 ng/ml	PARG 25ng/ ml	PARG 25 ng/ml	1 µM DEA + 50 ng/ml PARG	1 µM DEA + 50 ng/ml PARG	1 µM DEA + 50 ng/ml PARG	Cell/ Tissue Extract #2 10 µg Protein	Cell/ Tissue Extract #2 10 µg Protein	Cell/ Tissue Extract #2 10 µg Protein	Cell/ Tissue Extract #4 10 µg Protein	Cell/ Tissue Extract #4 10 µg Protein	Cell/ Tissue Extract #4 10 µg Protein
G	PARG 50 ng/ml	PARG 50 ng/ml	PARG 50 ng/ml	0.1 µM DEA + 50 ng/ml PARG	0.1 µM DEA + 50 ng/ml PARG	0.1 µM DEA + 50 ng/ml PARG	Cell/ Tissue Extract #2 5 µg Protein	Cell/ Tissue Extract #2 5 µg Protein	Cell/ Tissue Extract #2 5 µg Protein	Cell/ Tissue Extract #4 5 µg Protein	Cell/ Tissue Extract #4 5 µg Protein	Cell/ Tissue Extract #4 5 µg Protein
H	PARG 100 ng/ml	PARG 100 ng/ml	PARG 100 ng/ml	No DEA + 50 ng/ml PARG	No DEA + 50 ng/ml PARG	No DEA + 50 ng/ml PARG	Cell/ Tissue Extract #2 2 µg Protein	Cell/ Tissue Extract #2 2 µg Protein	Cell/ Tissue Extract #2 2 µg Protein	Cell/ Tissue Extract #4 2 µg Protein	Cell/ Tissue Extract #4 2 µg Protein	Cell/ Tissue Extract #4 2 µg Protein

Figure 3. Suggested layout for a PARG standard curve, PARG inhibitor serial dilutions, and for assay of PARG in cell extracts. Note that the histones on the plate must first be poly(ADP) ribosylated following Section VI.A.

IX. References

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X. Troubleshooting

PROBLEM	CAUSE	SOLUTION
High light output in wells with PARG Inhibitor at all inhibitor dilutions.	PARG inhibitor is extremely potent.	Increase the serial dilutions of your inhibitor.
High light output in wells with PARG alone in PARG inhibitor studies.	PARG enzyme was not added to the wells.	Add 25 µL of 100 ng/ml PARG to each well.
Low light output in wells containing cell extracts.	PARG expression in cells too high.	Increase the serial dilutions of your cell extracts in 1X PARG
High light output in PARG standard curve in wells with high concentrations of PARG	High background due to poor washing	Increase the number of washing steps.

XI. Related Products Available From Trevigen

Kits:

Catalog #	Description	Size
4683-096-K	HT Colorimetric PARG Assay Kit	96 tests
4520-096-K	HT PARP In Vivo Pharmacodynamic Assay II	96 tests
4690-096-K	HT F Homogeneous PARP Inhibition Assay Kit	96 tests
4676-096-K	HT Universal Chemiluminescent PARP Assay Kit/w Histone Coated Strip Wells	96 tests
4677-096-K	HT Universal Colorimetric PARP Assay Kit/w Histone Coated Strip Wells	96 tests
4684-096-K	HT Colorimetric PARP/Apoptosis Assay	96 tests
4685-096-K	HT Chemiluminescent PARP/Apoptosis Assay	96 tests

Inhibitors and Antibodies:

Catalog #	Description	Size
4667-50-11	Benzamide PARP inhibitor (8 mM)	100 µl
4667-50-03	3-Aminobenzamide PARP inhibitor (200 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
4335-AMC-050	PAR Monoclonal Antibody Affinity Purified	50 µl
4335-MC-100	Anti-PAR Monoclonal Antibody	100 µl
4336-APC-050	PAR Polyclonal Antibody Affinity Purified	50 µl
4336-BPC-100	Anti-PAR Polyclonal Antibody (rabbit)	100 µl
4338-MC-50	Anti-PARP Monoclonal Antibody (clone C2-10)	50 µg
2305-PC-100	Anti-Cleaved Caspase 3 Polyclonal	100 µl

Poly(ADP)-ribose:

Catalog #	Description	Size
4336-100-01	Poly(ADP) Ribose (PAR) Polymer	100 µl

PARG:

Catalog #	Description	Size
4680-096-01	PARG	100 µl

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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