

PARP in vivo Pharmacodynamic Assay

96 Tests

Cat# 4510-096-K

Pharmacodynamic PAR ELISA kit for quantifying poly(ADP-ribose) (PAR) in peripheral blood mononuclear cells and tissue culture cells

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I. Introduction

Inhibition of Poly (ADP-ribose) polymerase (PARP) promotes chemosensitization and radiosensitization of BRCA-1^{-/-} and BRCA-2^{-/-} tumor cells (deficient in homologous recombination),¹ and prevents tissue damage in animal models of myocardial and neuronal ischemia, diabetes, septic shock, and vascular stroke.²⁻⁹ ADP-ribosylation is a post-translational event that occurs in response to DNA damage. PARP catalyzes the NAD-dependent addition of poly (ADP-ribose) (PAR) onto itself and adjacent nuclear proteins. Furthermore, polymorphisms in the PARP-1 gene correspond to disease predisposition, and variation in PARP activity might have an impact on the effects of PARP inhibitors in clinical settings.⁷⁻⁹ To address the need to monitor PARP activity among different individuals, and within cells, Trevigen offers its validated **PARP in vivo Pharmacodynamic Assay** which measures net PAR levels in cellular extracts. This assay has also been used to document differences in PAR levels among tumor lysates from different tissues, organs and xenografts.^{10,11}

An immobilized PAR monoclonal antibody in the wells of a 96-well plate captures cellular PAR and PAR attached to proteins. Incubation with a polyclonal PAR Detecting Antibody PAR antibody, followed by goat anti-rabbit IgG-HRP secondary and a chemiluminescent HRP substrate yields relative light units (RLU) that directly correlates with the amount of cellular PAR. This assay is ideal for quantification of PAR in peripheral blood mononuclear cells and tissue culture cells, for monitoring the efficacy of PARP inhibitors on cellular PAR formation, and for verifying observations of enhanced cancer cell cytotoxicity arising from PARP inhibitor/anticancer drug combination therapy.^{4, 5, 10} Important features of the assay include: 1) chemiluminescent, non-radioactive format; 2) higher throughput 96 test size and, 3) sensitivity down to 10 pg/ml of PAR.

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 **PARP in vivo Pharmacodynamic Assay** 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	Storage Temperature
4500-096-01	PAR Standard, 6.25 pg/μl	50 μl	-20°C
4500-096-02	Blocking/Sample Buffer	30 ml	4°C
4500-096-03	PAR Monoclonal Capture Antibody	15 μl	-20°C
4500-096-04	PAR Polyclonal Detecting Antibody	30 μl	-20°C
4500-096-05	Goat anti-Rabbit IgG-HRP	30 μl	-20°C
4675-096-01	PARP PeroxyGlow™ A	6 ml	4°C
4675-096-02	PARP PeroxyGlow™ B	6 ml	4°C
4500-096-06	Cell Lysis Reagent	30 ml	4°C
4500-096-07	20% (w/v) SDS	1 ml	RT
4500-096-08	DNase I, 2 Units/ μl	30 μl	-20°C
4500-096-09	100X Magnesium Cation	500 μl	-20°C
4500-096-10	Antibody Coating Solution	10 ml	4°C
4510-096-P	White 96-stripwell plate, and 5 sealers	1plate	RT
4500-096-11	Jurkat Cell Lysate Standard	900 μl	-80°C
4500-096-12	Antibody Diluent	12 ml	4°C

IV. Materials/Equipment Required But Not Supplied

Reagents/Disposables:

1. Biological specimens to be tested
2. Tris-buffered saline containing 0.1% Tween 20 (TBST)
3. Distilled water
4. 200 mM Phenylmethyl Sulfonyl Fluoride (PMSF) in ethanol
5. Protease Inhibitor Cocktail (Thermo Fisher Scientific, Cat# 78429, optional)
6. 1 - 200 μl and 100-1000 μl pipette tips
7. PBS and/or Plasma Lyte A (Baxter HealthCare, Corporation)
8. Ficoll-Hypaque™ or Lymphocyte Separation Medium (Mediatech, Inc., cat# 25-072-CV).
9. Trypsin for detaching adherent cells

Equipment:

1. Micropipettes and tips
2. Multichannel pipettor 10 μl - 100 μl
3. Wash bottle or microstrip wells plate washer (optional)
4. Vacutainer™ tubes (BD, for blood collection)
5. 96-well chemiluminescent plate reader or luminometer
6. Refrigerated centrifuge with swinging bucket rotor
7. Microcentrifuge
8. 15 ml and 50 ml screw cap centrifuge tubes
9. 0.5 ml and 1.5 ml microtubes
10. 25 ml solution reservoirs

V. Reagent Preparation

1. TBS + 0.1% Tween 20 Wash Solution (TBST)

Prepare 500 ml of 1X TBST containing 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.1% Tween 20 in a wash bottle for washing the strip wells.

2. Preparation of PAR Monoclonal Capture Antibody-coated strip wells

The white strip wells (Cat# 4510-096-P) must be coated with PAR monoclonal capture antibody (Cat# 4500-096-03) the afternoon before performing the assay. Dilute the antibody 500-fold in Antibody Coating Solution (Cat# 4500-096-10) and add 50 μl per well with a multichannel pipettor. Cover the wells with sealing film and incubate overnight at 4°C. For coating a whole plate, dilute 12 μl of PAR monoclonal capture antibody into 6 ml of Antibody Coating Solution.

3. PAR Standard

The kit contains 50 μl of PAR standard (Cat# 4500-096-01) at a concentration of 6.25 pg/μl. Serially dilute the PAR standard with Blocking/Sample Buffer (Cat# 4500-096-12) just before use. The volume of each dilution should be 200 μl or greater. The recommended final concentrations are 6.25 pg/50 μl (125 pg/ml), 3.125 pg/50 μl (62.5 pg/ml), 1.5625 pg/50 μl (31.25 pg/ml), 0.625 pg/50 μl (12.5 pg/ml), and 0.3125 pg/50 μl (6.25 pg/ml). The standard curve requires 50 μl/well of each PAR dilution and each is performed in triplicate. **Note: Diluted PAR should be used immediately and any remainder discarded.** The following table describes a serial dilution protocol for PAR:

PAR Conc:	6.25 pg/well (125 pg/ml)	3.12 pg/well (62.5 pg/ml)	1.56 pg/well (31.25 pg/ml)	0.62 pg/well (12.5 pg/ml)	0.31 pg/well (6.25 pg/ml)
PAR Standard 6.25 pg/ μ l	8 μ l	200 μ l	200 μ l	160 μ l	200 μ l
Blocking Sample Buffer	392 μ l	200 μ l	200 μ l	240 μ l	200 μ l

4. PAR Polyclonal Detecting Antibody

One to two hours before use, dilute the PAR polyclonal detecting antibody (Cat# 4500-096-04) 250-fold with Antibody Diluent (Cat# 4500-096-12). A total of 50 μ l/well of diluted PAR polyclonal antibody is required in the assay. For example, for a whole plate, dilute 24 μ l of PAR polyclonal detecting antibody into 6 ml of Antibody Diluent and add 50 μ l/well with a multichannel pipettor.

5. Goat Anti-Rabbit IgG-HRP Conjugate

One to two hours before use, dilute the Goat anti-Rabbit IgG-HRP conjugate (Cat# 4500-096-05) 250-fold with Antibody Diluent (Cat# 4500-096-12). A total of 50 μ l/well of diluted Goat anti-Rabbit-HRP conjugate is required in the assay. For example, for a whole plate, dilute 24 μ l of Goat anti-Rabbit IgG-HRP conjugate into 6 ml of Antibody Diluent and add 50 μ l/well with a multichannel pipettor.

6. PARP PeroxyGlow™ A and B Chemiluminescent Substrates

Allow PARP PeroxyGlow A and B to come to room temperature. Just before use, mix equal volumes of PARP PeroxyGlow™ A and B together. A total of 100 μ l is required per well. PARP PeroxyGlow™ A and B are Horseradish Peroxidase (HRP) substrates generating light that can be quantified in a suitable chemiluminescent plate reader. For example, for a whole plate, mix 6 ml of PARP PeroxyGlow A with 6 ml of PARP PeroxyGlow B together and add 100 μ l/well with a multichannel pipettor.

7. Cell Lysis Buffer

Just before use, prepare 1 ml of the following Cell Lysis Buffer and store at 4°C:

Cell Lysis Reagent (Cat# 4500-096-06)	985 μ l
200 mM PMSF (in ethanol)	5 μ l
100X Protease Inhibitor Cocktail (Thermo Scientific, Cat# 78429, optional)	10 μ l

8. 20% SDS

The SDS may have precipitated out of solution during shipping. To solubilize the SDS, warm the tube at 37°C for 10 minutes and vortex periodically. Store the SDS solution at room temperature.

9. Jurkat Cell Lysate Standard

This standard measures assay drift between different experiments and serves as means of validating the assay. The kit contains one vial of Jurkat Cell Lysate Standard (Cat# 4500-096-11) at a PAR concentration of 80

pg/ml \pm 15 pg/ml and is ready for use. We recommend that you run undiluted extract and a 1:4 dilution of the extract (diluted with blocking/Sample Buffer) in triplicate wells each with each experiment. There is sufficient standard for three experiments. Store the lysate at -80°C in working 300 μ l aliquots and keep on ice after thawing.

VI. Preparation of Cell Extracts

i. Suspension cells:

1. Grow 2-10 x 10⁶ suspension cells in complete medium in a suitable tissue culture plate or flask.
2. Transfer the cells to prechilled 15 ml screw cap tubes. Count the cells and then centrifuge at 250 x g for 5 min at 4°C. Discard the supernatant. Wash the cells one more time with 10 ml of ice-cold 1X PBS.
3. Suspend the cell pellets in 1 ml of ice-cold 1X PBS. Transfer to 1.5 ml microtubes and centrifuge at 10,000 x g or top speed in a microcentrifuge for 10 sec at 4°C. Discard the supernatant.
4. Resuspend the cell pellet at a cell concentration of 1-5 x 10⁷ cells/ml in Cell Extraction Buffer (Section V.7). Incubate the cell suspensions on ice, with periodic vortexing, for 5 minutes.
5. Add 20% SDS (Cat# 4500-096-07) to a final concentration of 1%. For example, add 50 μ l of 20% SDS to 950 μ l of resuspended cells.
6. Incubate cell extract at 100°C for 5 minutes. Cool to room temperature.
7. Add 0.01 volume of 100X Magnesium Cation (Cat# 4500-096-09) and 2 μ l of DNase I (2 Units/ μ l, Cat# 4500-096-08). Vortex briefly and incubate at 37°C for 90 minutes. This step degrades cellular DNA and reduces the viscosity of the extract.
8. Centrifuge at 10,000 x g for 10 minutes at room temperature to remove cellular debris. Remove the pellet with a toothpick or pipette tip. Save the supernatant.
9. Measure the protein concentration of the extracts with a BCA protein assay.
10. Assay immediately for PAR or aliquot the extracts and store at -80°C.

ii. Adherent cells:

1. Grow 2-10 x 10⁶ adherent cells in a suitable tissue culture 60 mm dish or 6 well plate in complete medium until 75% confluent.
2. Remove the medium and gently wash the cells with 5 ml of warm (37°C) PBS. Carefully pipette out the PBS. Repeat this step one more time.

5. Add 300 µl of cold Cell Extraction Buffer (Section V.7) to each well of a 6 well plate, or 500 µl to a 60 mm dish. Place the dish or plate on ice and immediately scrape the cells with a cell scraper to detach the cells. Incubate the cell suspensions on ice, with periodic scraping, for 5 minutes.
6. Transfer the cell suspensions to 1.5 ml tubes. Add 20% (w/v) SDS (Cat# 4500-096-07) to a final SDS concentration of 1%. For example, add 50 µl of 20% SDS to 950 µl of resuspended cells.
7. Incubate cell extract at 100°C for 5 minutes. Cool to room temperature.
8. Add 0.01 volume of 100X Magnesium Cation (Cat# 4500-096-09) and 2 µl of DNase I (2 Units/µl, Cat# 4500-096-08). Vortex briefly and incubate at 37°C for 90 minutes. This step degrades cellular DNA and reduces the viscosity of the extract.
9. Centrifuge at 10,000 x g for 10 minutes at room temperature. Remove the pellet with a toothpick or pipette tip.
10. Measure the protein concentration of the extracts with a BCA protein assay.
11. Assay immediately for PAR or aliquot the extracts and store at -80°C

iii. Peripheral Blood Mononuclear Cells (PBMC)

Note: It is important to make the PBMC lysates as quickly as possible after blood drawing to avoid cellular degradation of PAR.

1. Withdraw 20 ml of blood into two purple-top Vacutainer™s containing EDTA as anticoagulant. Dilute the blood with an equal volume of Plasma Lyte A (Baxter Healthcare). In each of two 50 ml conical centrifuge tubes, carefully layer the diluted blood over 10 ml of Ficoll-Hypaque® or Lymphocyte Separation Medium.
2. Centrifuge in a swinging bucket rotor at 1000 x g for 30 minutes at 4°C. Recover the layer of PBMCs between the plasma and Ficoll-Hypaque® interface and transfer to a clean 50 ml centrifuge tube.
3. Fill the tube containing PBMCs with cold Plasma Lyte A to the 50 ml mark and centrifuge at 250 x g for 10 minutes at 4°C. Discard the supernatant.
4. Suspend the cell pellet in cold Plasma Lyte A and fill to the 50 ml mark.
5. Count the PBMCs in a hemocytometer. Centrifuge at 250 x g for 10 minutes at 4°C. Discard the supernatant.
6. Suspend the cell pellet in 1 ml of cold Plasma Lyte A and transfer to a 1.5 ml microtube. Keep it on ice.

7. Centrifuge at 10,000 x g or top speed for 10 sec at 4°C. Discard the supernatant.
8. Resuspend the cells at a concentration of 5×10^7 cells/ml in cold Cell Extraction Buffer (Section V.7). Incubate the cell suspensions on ice, with periodic vortexing, for 5 minutes.
9. Add 20% SDS (Cat# 4500-096-06) to a final concentration of 1%. Vortex well. For example: add 50 µl of 20% SDS to 950 µl of resuspended cells.
10. Incubate cell extract at 100°C for 5 minutes. Cool to room temperature.
11. Add 0.01 volume of 100X Magnesium Cation (Cat# 4500-096-09) and 2 µl of DNase I (2 Units/µl, Cat# 4500-096-08). Vortex briefly and incubate at 37°C for 90 minutes. This step degrades cellular DNA and reduces the viscosity of the extract.
12. Centrifuge at 10,000 x g for 10 minutes at room temperature. Remove the pellet with a toothpick or pipette tip.
13. Assay immediately for PAR or aliquot the extracts and store at -80°C.

VII. Assay Protocol

1. The day before performing the assay, coat the required number of strip wells with PAR monoclonal capture antibody as described in **Section V.2**.
2. The next morning, wash the wells 4 times with 200 µl/well TBST. Tap the plate on dry paper towels between each wash.
3. Add 100 µl/well of Blocking/Sample Buffer to block the strip wells. Cover the wells with sealing film. Incubate at room temperature for 1 hour. During this time, make serial dilutions of the PAR standard (**Section V.3**) and test samples with Blocking/Sample Buffer. Include three wells for undiluted Jurkat Cell Lysate Standard, and three wells for a 1:4 dilution of Jurkat Cell Lysate Standard. Samples and PAR standard are assayed in triplicate and require $3 \times 50 \mu\text{l/well} = 150 \mu\text{l}$ minimum volume. A total of 200 µl is recommended. Include three wells with 50 µl/well of Antibody Diluent to act as background wells.

NOTES:

- i. The extracts must be diluted at least 3- to 5-fold with Sample/Blocking Buffer to reduce the SDS concentration to below 0.33%.
- ii. The assay can detect a minimum of 0.5 pg/well of PAR in 50 µl (10 pg/ml). If the sample is diluted 5-fold, the assay can detect a minimum of 50 pg/ml (10 pg/ml x 5 fold dilution) of PAR in the original sample.
- iii. The assay requires diluted cell extract equivalent to 1,000-500,000 cells/well, depending on the cell type. Tumor cell lines such as MCF7

may contain 1000 pg of PAR per 10^6 cells, whereas PBMCs may contain less than 40 pg PAR/ 10^6 cells. If the extract is at a “cell concentration” of 5×10^7 cells/ml, and if the extract is then diluted 5-fold with Sample/Blocking Buffer, then the number of “cells” per well is calculated as follows:

$$\begin{aligned} \text{“Cells/well”} &= \frac{\text{Original Cell Concentration}}{\text{Dilution Factor}} \times 0.05 \text{ ml/well} \\ &= \frac{5 \times 10^7 \text{ cells/ml}}{5} \times 0.05 \text{ ml/well} \\ &= 500,000 \text{ cells/well} \end{aligned}$$

- iv. The assay requires diluted PBMC extract equivalent to at least 250,000 cells/well, although 500,000 cells/well is preferred.
 - v. It is best to report PAR in terms of pg/ml in the PBMC extract or as pg PAR per 10^6 PBMC rather than pg PAR/mg protein. The reliability of the protein content may be problematic because of adherence and carry-over of plasma proteins to the surface of some PBMC.
 - vi. PAR levels in suspension and adherent cell lines may be reported either as pg/ml per 10^6 cells or pg PAR/mg extract protein.
4. Remove the Blocking/Sample Buffer from the wells by tapping the strip wells on paper towels. Add 50 μ l/well of the serial dilutions of PAR standard (Section V.3) and 50 μ l/well of the cell extracts to appropriate wells in triplicate.
 5. Cover the wells with sealing film and incubate the strip wells for 2 hours at room temperature.
 6. Gently remove the plate sealer and wash strip wells 4 times with TBST (200 μ l/well). Ensure that all the liquid is removed following each wash by tapping strip wells onto paper towels.
 7. Add 50 μ l per well of diluted PAR polyclonal detecting antibody (prepared in section V.4). Cover the wells with sealing film and incubate at room temperature for 2 hours. **Note that it is important to dilute the PAR polyclonal detecting antibody 1:250 fold in Antibody Diluent at least 1 hour before use.**
 8. Gently remove the plate sealer and wash strip wells 4 times with TBST (200 μ l/well). Ensure that all the liquid is removed following each wash by tapping strip wells onto paper towels.
 9. Add 50 μ l per well of diluted Goat anti-Rabbit IgG-HRP conjugate (prepared in section V.5). Cover the wells with sealing film and incubate at room temperature for 1.5 hour. **Note that it is important to dilute the conjugate in Antibody Diluent 1:250 fold at least 1 hour before use.** During this

time bring the PARP PeroxyGlow A and B reagents to the work bench and allow to warm to room temperature.

10. Gently remove the plate sealer and wash strip wells 4 times with TBST (200 μ l/well). Ensure that all the liquid is removed following each wash by tapping strip wells onto paper towels.
11. Just before use, mix equal volumes of room-temperature PeroxyGlow™ A and B together and add 100 μ l per well. **Immediately** take chemiluminescent readings.

VIII. Data Interpretation

Subtract the mean background Relative Light Units (RLU) from the mean RLU values of the PAR standards and of the cell extracts. This calculation determines the net mean RLU. Plot the net mean RLU data of the PAR standards as described in **Figure 1**.

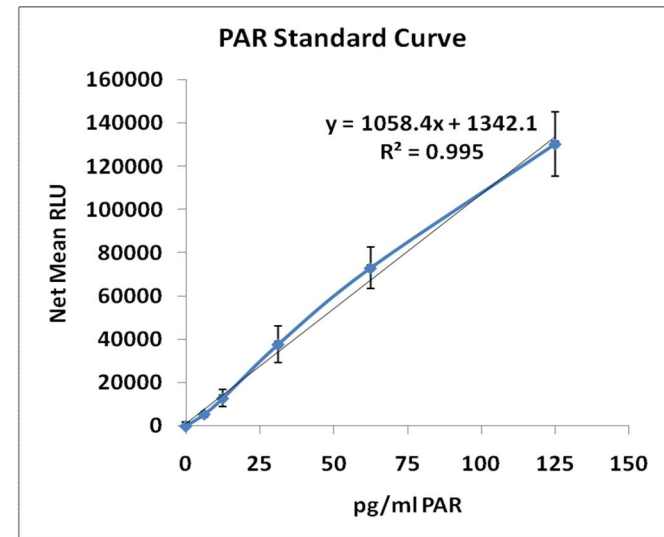


Figure 1. PAR standard curve with trend line. Shown is the mean of three experiments \pm standard deviation.

Determine the levels of PAR in your cell extract from the standard curve. Express the results as pg PAR/ml in your original extract, pg PAR/ 10^6 cells, or pg PAR/mg extract protein in cell lines.

Levels of PAR in untreated Jurkat cells and Jurkat cells treated with PJ34, a potent PARP inhibitor, and with PARG, are graphically represented in **Figure 2**.

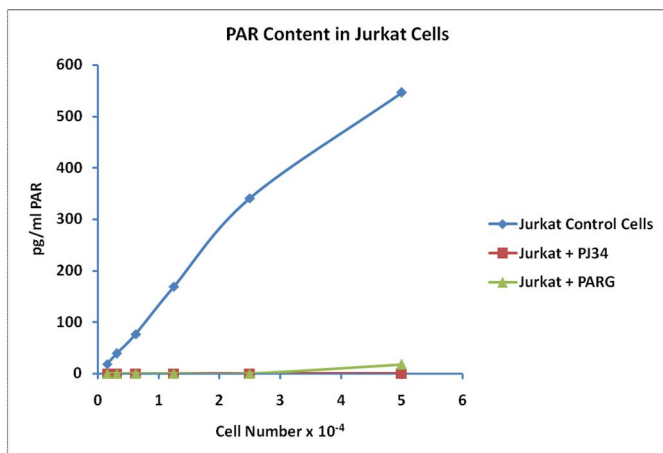


Figure 2. Graphical representation of PAR in Jurkat cells exposed to PJ34, a potent PARP inhibitor, and to PARG. Jurkat cells were exposed to 1 μ M PJ34 for 1.5 hr and then harvested (PJ34 was included in the Cell Lysis Buffer). Extracts were prepared as described. PARG (10 ng) was added to the Jurkat cells during the lysis procedure and incubated at room temperature for 30 minutes prior to the addition of SDS. The lysates were then processed as described. Control Jurkat cells are untreated.

The content of PAR in PBMCs from normal subjects and in the Jurkat Cell Lysate Standard is illustrated in **Figure 3**:

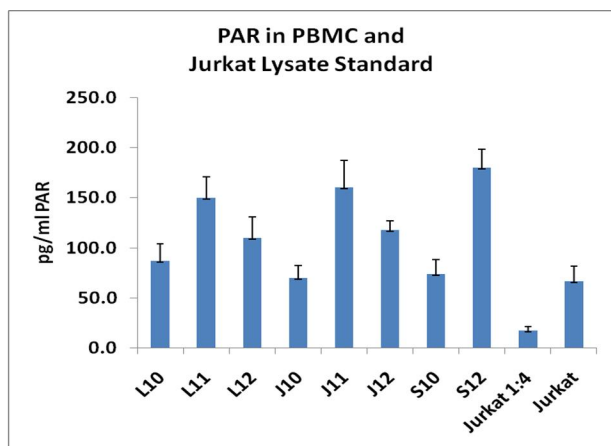


Figure 3. Graphical representation of PAR content in PBMCs from three subjects (L, J, and S) collected over a 3 week period, the Jurkat Cell Lysate Standard, and a 1:4 dilution of the Jurkat Cell Lysate Standard. Each was assayed three times and standard deviation of three experiments shown.

IX. References

1. Cao WH, Wang X, Frappart L, Rigal D, Wang ZQ, Shen Y, Tong WM. 2007. Analysis of genetic variants of the poly(ADP-ribose) polymerase-1 gene in breast cancer in French patients. *Mutat Res* **632**:20-8.
2. Piper, A.A., A.Verma, J. Zhang, and S.H. Snyder. 1999. Poly(ADP-ribose) polymerase, nitric oxide and cell death. *Trends in Pharmacological Sciences* **20**:171-81.
3. Thiernemann, C., J. Bowes, F.P. Myint, and J.R. Vane. 1997. Inhibition of the activity of poly(ADP-ribose) synthase reduces ischemia-reperfusion injury in the heart and skeletal muscle. *Proc Natl Acad Sci USA* **94**:679-83.
4. Virag, L., and Szabo, C. 2002. The therapeutic potential of Poly(ADP-Ribose) Polymerase inhibitors. *Pharmacological Reviews* **54**:375-429.
5. Curtin NJ. 2005. PARP inhibitors for cancer therapy. *Expert Rev Mol Med* **7**:1-20.
6. Hur JW, Sung YK, Shin HD, Park BL, Cheong HS, Bae SC. 2006. Poly(ADP-ribose) polymerase (PARP) polymorphisms associated with nephritis and arthritis in systemic lupus erythematosus. *Rheumatology* **45**:711-7.
7. Infante J, Sánchez-Juan P, Mateo I, Rodríguez-Rodríguez E, Sánchez-Quintana C, Llorca J, Fontalba A, Terrazas J, Oterino A, Berciano J, Combarros O. 2007. Poly(ADP-ribose) polymerase-1 (PARP-1) genetic variants are protective against Parkinson's disease. *J Neurol Sci* **256**:68-70.
8. Gao R, Price DK, Sissung T, Reed E, Figg WD. 2008. Ethnic disparities in Americans of European descent versus Americans of African descent related to polymorphic ERCC1, ERCC2, XRCC1, and PARP1. *Molecular Cancer Therapeutics* **7**:1246-50.
9. Nikitin AG, Chudakova DA, Stokov IA, Bursa TR, Chistiakov DA, Nosikov VV. 2008. Leu54Phe and Val762Ala polymorphisms in the poly(ADP-ribose) polymerase-1 gene are associated with diabetic polyneuropathy in Russian type 1 diabetic patients. *Diabetes Res Clin Pract* **79**:446-52.
10. Liu X, Palma J, Kinders R, Shi Y, Donawho C, Ellis PA, Rodriguez LE, Colon-Lopez M, Saltarelli M, LeBlond D, Lin CT, Frost DJ, Luo Y, Giranda VL. 2008. An enzyme-linked immunosorbent poly(ADP-ribose) polymerase biomarker assay for clinical trials of PARP inhibitors. *Anal Biochem* **381**:240-7.
11. Miknyoczki SJ, Jones-Bolin S, Pritchard S, Hunter K, Zhao H, Wan W, Ator M, Bihovsky R, Hudkins R, Chatterjee S, Klein-Szanto A, Dionne C, Ruggeri B. 2003. Chemopotentiation of Temozolomide, Irinotecan, and Cisplatin Activity by CEP-6800, a Poly(ADP-Ribose) Polymerase Inhibitor. *Mol Cancer Ther* **2**:371-82.

X. Troubleshooting

PROBLEM	CAUSE	SOLUTION
No relative light units (RLU) in experimental sample wells but RLU are present in wells with the PAR standard	PAR levels in the samples are below the sensitivity of the assay.	Increase the number of "cell equivalents" added to each well.
	PARG activity in the cell extract is very high	Add ADP-HPD (1 μ M), a potent PARG inhibitor, to the Cell Lysis Buffer Check that specimen processing included addition of SDS and the boiling step; prepare a new specimen or add SDS and boil existing specimen.

PROBLEM	CAUSE	SOLUTION
No RLU in wells containing PAR standard	PAR standards were not added to the wells	Add serial dilutions of PAR standard to appropriate triplicate wells
RLU in wells containing cell or tissue extracts too high or above that obtained for the PAR standard curve	PAR levels in cells and tissues very high	Extend serial dilutions of extract down to 1,000 "cells" per well. At higher dilutions, results should be checked for linearity by back-calculating pg/PAR/mL per cell number or micrograms protein. Expect non-linearity to appear at the highest dilutions, and choose a value such as the Mean for the linear range; or alternatively, use the first dilution value that plots on the standard curve.
High background in wells with no PAR	Poor washing	Increase the number of washes with TBST between steps.
	PAR Detecting Antibody concentration too high	Increase dilution of PAR Detecting Antibody from 1:250 to 1:500
	Failure to pre-absorb rabbit anti-PAR with BSA in the Antibody Diluent	Retest specimens following the preincubation steps in the protocol (see step VII.7 and VII.9)
High variability within triplicates	Uneven distribution of reagents	Check quality of single and multichannel pipettors
		Practice repetitive pipetting technique
	If 2 of 3 replicates agree, the third may be tested for inclusion/exclusion by Dixon's Rule	
Incomplete solubilization and clarification of the specimen	Check specimen for viscosity (indication of large quantity of intact DNA) and particulates. Repeat the DNase I treatment and centrifugation steps	
Assay Controls out of range high or low	Uneven distribution of reagents	Repeat assay
Excessive variability in assay controls (>20%)	Poor washing uneven distribution of reagents improper storage and handling failure to pre-incubate probe	Repeat assay; if only one control is out of range, do not use specimen values that plot to the same segment of the standard curve; specimens plotting in the standard curve segment of valid controls may be used

PROBLEM	CAUSE	SOLUTION
Lower limit of quantitation (LLQ) fails because low standard is within 2 SDEVs of the Mean Zero Read	Multiple causes: Poor washing Failure to preincubate Detecting Antibody Expired Expired standards Expired conjugate	Check package insert for expiration dates Check performance of plate washer Perform maintenance on plate washer Check handling of PAR Detecting Antibody and conjugate for performance of the preincubation step

XI. Related Products Available From Trevigen

Kits:

Catalog #	Description	Size
4671-096-K	HT Universal Color PARP Assay Kit/w Histone Reagents	96 samples
4675-096-K	Universal Chemiluminescent PARP Assay Kit/w Histone Reagent	96 samples
4676-096-K	Universal Chemiluminescent PARP Assay Kit/w Histone Coated Strip Wells	96 samples
4677-096-K	Universal Colorimetric PARP Assay Kit/w Histone Coated Strip Wells	96 samples
4684-096-K	HT Colorimetric PARP Apoptosis Assay	96 samples
4685-096-K	HT Chemiluminescent PARP Apoptosis Assay	96 samples
4690-096-K	HT F Homogeneous PARP Inhibition Assay Kit	96 tests
4682-096-K	HT Chemiluminescent PARG Assay Kit	96 tests
4683-096-K	HT Colorimetric PARG Assay Kit	96 tests

Inhibitors and Antibodies:

Catalog #	Description	Size
4667-50-11	Benzamide PARP inhibitor (8 mM)	100 µl
4667-50-03	3-Aminobenzamide (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-50	PAR Monoclonal Antibody Affinity Purified	50 µl
4335-MC-100	Anti-PAR Monoclonal Antibody	100 µl
4336-APC-50	PAR Polyclonal Antibody Affinity Purified	50 µl
4336-BPC-100	Anti-PAR Polyclonal Antibody (rabbit)	50 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

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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