TREVIGEN® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

HT PARP in vivo Pharmacodynamic Assay II

96 Tests

Cat# 4520-096-K

High throughput chemiluminescent ELISA to quantify polyADP-ribose (PAR) in peripheral blood mononuclear cells, tissues, and cultured cells with pre-coated plate

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Table of Contents

I. Introduction

In response to DNA damage, poly-(ADP-ribose) polymerase-1 (PARP-1), which is the main isoform of the PARP family, is rapidly activated by DNA strand breaks occurring from exposure to environmental agents, cancer therapy, inflammation, ischemia-reperfusion and neurodegeneration.[1] Once activated, NAD^+ is consumed for the synthesis of the highly negatively charged polymer poly-ADP-ribose (PAR), which is found on target nuclear proteins including PARP-1 as a major acceptor. These highly branched polymers are in turn rapidly degraded by poly- (ADP-ribose) glycohydrolase (PARG). As a consequence of PARP activation, extensive DNA damage can lead to the depletion of NAD^+ in the cell, and lead to cell death. Therefore, PARP-1 is regarded as a promising target for the development of drugs useful in various regimens of cancer therapy, inflammation, ischemia and neurodegeneration.[1-3] The discovery that breast cancers deficient in homologous recombination are sensitive to nontoxic PARP inhibitors, has resulted in efforts by numerous pharmaceutical companies to develop PARP-1 specific drugs.

To address the need to monitor PARP activity among different individuals, and within cells, Trevigen offers its' validated **HT PARP in vivo Pharmacodynamic Assay II** which measures net PAR levels in cellular extracts. This assay employs a validated sample processing procedure and has been used to document differences in PAR levels among PBMC cells and tumor lysates from different tissues, organs and xenografts.[4]

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, followed by addition of a 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, tissues, and cultured cells. Additional uses are 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.[3] Important features of the assay include: 1) chemiluminescent, non-radioactive format; 2) high throughput 96 test format with pre-coated capture antibody; 3) broad linear dynamic range to 1000 pg/ml; and, 4) high signal to noise ratio with increased sensitivity at 2 pg/ml of PAR.

Trevigen's **HT PARP in vivo Pharmacodynamic Assay II** employs a purified, pre-coated monoclonal PAR antibody as the capture agent, and anti-PAR polyclonal rabbit antibody as the detecting agent. Kit components were subjected to a published validation protocol including procedures facilitating the analysis of human tumor xenografts. Please see: Kinders, et al., *Clin Cancer Res* **14**(21): 6877-85 '08.

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 PARP in vivo Pharmacodynamic Assay II** 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
4520-096-01	PAR Standard, 25 pg/µl	5 x 20 µl	-80°C
4520-096-02	Sample Buffer	20 ml	4°C
4520-096-03	PAR Polyclonal Detecting Antibody	30 µl	-20°C
4520-096-04	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
4520-096-05	Cell Lysis Reagent	30 ml	4°C
4520-096-06	DNase I, 2 Units/ µl	60 µl	-20°C
4520-096-07	100X Magnesium Cation	500 µl	4°C
4520-096-P	Pre-coated white 96-stripwell plate, and 5 sealers	1 plate	4°C
4520-096-08	Jurkat Cell Lysate Standard Control, Low	600 µl	-80ºC
4520-096-09	Jurkat Cell Lysate Standard Control, Medium	600 µl	-80ºC
4520-096-10	Jurkat Cell Lysate Standard Control, High	600 µl	-80ºC
4520-096-11	Antibody Diluent	15 ml	4°C
4520-096-12	20% (w/v) SDS	1 ml	RT

IV. Materials/Equipment Required But Not Supplied

Reagents/Disposables:

1. Biological specimens to be tested
2. 1XPBS-buffered saline containing 0.1% Tween 20 (PBST)
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, Corp. cat# 2B2544X)
8. 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. BD Vacutainer® CPT™ (Na HEPARIN) (Ref 362753)
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
11. Incubator set at 25 ºC

V. Reagent Preparation

1. PBS + 0.1% Tween 20 Wash Solution (PBST)

Prepare 500 ml of 1X PBST containing 1X PBS and 0.1% Tween 20 in a wash bottle for washing the strip wells.

2. Pre-coated capture antibody strip wells

Pre-coated anti-PAR monoclonal white strip wells (cat# 4520-096-P) are provided for convenience.

3. PAR Standard

The kit contains 5 vials (20 µl/vial) of PAR standard (cat#: 4520-096-01) at a concentration of 25 pg/µl. Thaw each aliquot only once. Serially dilute the PAR standard with Sample Buffer (cat# 4520-096-02) just before use. The volume of each dilution should be 200 µl or greater. The recommended final concentrations are 1000 pg/ml, 500 pg/ml, 200 pg/ml, 100 pg/ml, 50 pg/ml, 20 pg/ml and 10 pg/ml. The standard curve requires 50 µl/well of each PAR dilution and each is performed in triplicate. The following table describes a serial dilution protocol for PAR:

PAR Conc.	1000 pg/ml (50 pg/well)	500 pg/ml (25 pg/well)	200 pg/ml (10 pg/well)	100 pg/ml (5 pg/well)	50 pg/ml (2.5 pg/well)	20 pg/ml (1 pg/well)	10 pg/ml (0.5 pg/well)
PAR Standard 25 pg/µl	16 µl	200 µl	160 µl	200 µl	200 µl	160 µl	200 µl
Sample Buffer	384 µl	200 µl	240 µl	200 µl	200 µl	240 µl	200 µl

Note: Diluted PAR should be used immediately and any remainder discarded.

4. PAR Polyclonal Detecting Antibody

One hour before addition (Section VII step 6), dilute the PAR polyclonal detecting antibody (cat# 4520-096-03) 250-fold with Antibody Diluent (cat# 4520-096-11). A total of 50 µl/well of diluted PAR polyclonal antibody is required in the assay. For example, for a whole plate, dilute 24 µl of PAR polyclonal detecting antibody into 6 ml of Antibody Diluent and add 50 µl/well with a multichannel pipettor.

5. Goat Anti-Rabbit IgG-HRP Conjugate

One hour before addition (Section VII step 8), dilute the Goat anti-Rabbit IgG-HRP conjugate (cat# 4520-096-04) 250-fold with Antibody Diluent (cat# 4520-096-11). A total of 50 µl/well of diluted Goat anti-Rabbit-HRP conjugate is required in the assay. For example, for a whole plate, dilute 24 µl of Goat anti-Rabbit IgG-HRP conjugate into 6 ml of Antibody Diluent and add 50 µl/well with a multichannel pipettor.

6. PARP PeroxyGlow™ A and B Chemiluminescent Substrates

Allow PARP PeroxyGlow**™** A and B to come to room temperature before use. Immediately before addition (Section VII step 10), mix equal volumes of PARP PeroxyGlow™ A and B together. A total of 100 µl 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 µl/well with a multichannel pipettor.

7. Cell Lysis Buffer

Just before use, prepare 1 ml of the following Cell Lysis Buffer and place on ice:

Cell Lysis Reagent (cat# 4520-096-05)	985 µl
200 mM PMSF (in ethanol)	5 µl
100X Protease Inhibitor Cocktail (Thermo Scientific, cat# 78429, optional)	10 µl

8. 20% SDS

The 20% SDS (cat# 4520-096-12) may precipitate during shipping. To solubilize, warm the tube at 37ºC for 10 minutes and gently vortex periodically. Store the SDS solution at room temperature.

9 Jurkat Cell Lysate Standards

Three lysate standards, used to monitor assay drift between experiments, contain PAR levels at 750-1150 pg/ml (High, cat# 4520-096-10), 150-300 pg/ml (Medium, cat# 4520-096-09) and 20-60 pg/ml (Low, cat# 4520-096-08) which are ready for use. There are sufficient standards for three experiments. Store each lysate at -80ºC in working 200 µl aliquots and keep on ice after thawing.

VI. Preparation of Cell Extracts

***i. Suspension cells**:*

1. Grow 2-10 x 10^6 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 10^7 cells/ml in Cell Lysis Buffer (Section **V** item **7**). Incubate the cell suspensions on ice, with periodic vortexing, for 15 minutes.

5. Add 20% SDS (cat# 4520-096-12) to a final concentration of 1%. For example, add 50 µl of 20% SDS to 950 µl of resuspended cells.

6. Incubate cell extracts at 100ºC for 5 minutes. Cool to room temperature.

7. Add 0.01 volume of 100X Magnesium Cation (cat# 4520-096-07) and 2 µl of DNase I (2 Units/µl, cat# 4520-096-06). 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 10^6 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.

3. Add 300 µl of cold Cell Lysis Buffer (Section **V** item **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 15 minutes.

4. Transfer the cell suspensions to 1.5 ml tubes. Add 20% (w/v) SDS (cat# 4520-096-12) to a final SDS concentration of 1%. For example, add 50 µl of 20% SDS to 950 µl of resuspended cells.

5 Incubate cell extract at 100ºC for 5 minutes. Cool to room temperature.

6. Add 0.01 volume of 100X Magnesium Cation (cat# 4520-096-07) and 2 µl of DNase I (2 Units/µl, cat# 4520-096-06). Vortex briefly and incubate at 37°C for 90 minutes. This step degrades cellular DNA and reduces the viscosity of the extract.

7. Centrifuge at 10,000 x g for 10 minutes at room temperature. Remove the pellet with a toothpick or pipette tip.

8. Measure the protein concentration of the extracts with a BCA protein assay.

9. 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 8 ml of blood into one BD Vacutainer® CPT™ tube containing Sodium Heparin as the anticoagulant. Remix the blood sample by gently inverting the tube 8-10 times and then centrifuge at room temperature (18-25 ºC) in a horizontal rotor (swinging bucket) and a proper adaptor for 25 minutes at 1500 x g.

2. After centrifugation, the PBMCs are in a whitish layer under the plasma layer. Carefully transfer the PBMCs into 50 ml conical tube.

3. Add cold Plasma Lyte A to the PBMCs in the 50 ml tube to bring the volume up to 45 ml. Cap and mix by inverting 5-8 times.

4. Centrifuge at 330 x g for 10 minutes at 4 ºC. Discard the supernatant.

5. Resuspend the cell pellet in cold Plasma Lyte A and fill to the 10 ml mark.

6. Count the PBMCs in a hemocytometer. Centrifuge at 330 x g for 10 minutes at 4 °C. Discard the supernatant.

7. Resuspend the cell pellet in 1 ml of cold Plasma Lyte A and transfer to a 1.5 ml microtube. Keep it on ice.

8. Centrifuge at 10,000 x g or top speed for 10 sec at 4°C. Discard the supernatant.

9. Resuspend the cells at a concentration of 2 x 10^7 cells/ml in cold Cell Lysis Buffer (Section **V** item 7). Incubate the cell suspension on ice, with periodic vortexing, for 15 minutes.

10. Add 20% SDS (cat# 4520-096-12) to a final concentration of 1%. Vortex well. For example: add 50 µl of 20% SDS to 950 µl of resuspended cells.

11. Incubate cell extract at 100 ºC for 5 minutes. Cool to room temperature.

12. Add 0.01 volume of 100X Magnesium Cation (cat# 4520-096-07) and 2 µl of DNase I (2 Units/µl, cat# 4520-096-06). Vortex briefly and incubate at 37°C for 90 minutes. This step degrades cellular DNA and reduces the viscosity of the extract.

13. Centrifuge at 10,000 x g for 10 minutes at room temperature. Remove the pellet with a toothpick or pipette tip.

14. Assay immediately for PAR or aliquot the extracts and store at -80°C.

iv. Biopsies of tumor xenografts

1. Anesthetize host, and when unresponsive to toe pinch, disinfect incision area with Nolvasan (QC Supply cat# 540561).

2. Make a 2-5 mm incision adjacent to the tumor or cut a flap to expose it and insert a Temno 18 gauge biopsy needle (Cardinal Health cat# T186) into the tumor until fully perforated. Collect biopsy, place in Sarstedt cryovial and flash freeze in liquid nitrogen (this is important for stabilizing PAR levels). **Notes:** 1) Specimen sizes typically range from 5- 20 mm in length, and 3 to 12 mg in mass. 2) Refer to Kinders, et al., Clin Cancer Res 14(21):6877-85 ’08.

3. Add 0.5 ml Lysis Buffer (section **V** item **7**) to the frozen tissue and mince completely with fine-point scissors. Vortex to mix and place on ice.

4. Disrupt the extracts by sonication on ice three times for 10 seconds each cycle. Vortex and allow to stand on ice for 15 min.

5. Move samples to room temperature and add 20% SDS (cat# 4520-096-12) to a final concentration of 1% (e.g., add 25 µL 20% SDS into 475 µL lysate).

6. Vortex again and incubate at 100ºC for 5 minutes. Snap-cool on ice for 1 min.

7. Centrifuge at 10,000 x g for 2 minutes at 4 ºC. Collect the supernatant as a xenograft tumor lysate for each sample.

8. Measure the protein concentration of the extract with a BCA protein assay.

9. Assay immediately for PAR concentration or aliquot the extracts and store at -80 ºC.

VII. Assay Protocol

1. Remove the pre-coated stripwells from the foil pouch. **Note:** If less than 96 pre-coated wells are needed, remove the excess wells from the frame and return them to the foil pouch. Reseal the pouch containing the unused wells and desiccants, and store at 4 °C.

2. Make serial dilutions of the PAR standard (**Section V** item **3**) and test samples with Sample Buffer (cat# 4520-096-02). Samples and PAR standards are assayed in triplicate and require 3 x 50 µl/well = 150 µl minimum volume. A total of 200 µl is recommended.

NOTES:

i. The extracts must be diluted at least 3- to 5-fold with Sample Buffer to reduce the SDS concentration to below 0.33%.

ii. It is best to report PAR in terms of pg/ml in the PBMC extract or as pg PAR per 10^7 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.

iii. PAR levels in suspension and adherent cell lines may be reported either as pg PAR per 10^7 cells or pg/mg protein extract.

iv. Xenograft cell extracts are added in the range of 100 ng to 2000 ng/well using Sample Buffer. PAR levels in tumor xenograft cell extracts may be reported as pg PAR per 100 µg of protein extract.

3. Add 50 µl/well of the serially diluted PAR standards (Section **V** item **3**), diluted test samples, Jurkat Cell Lysate Standards (Section **V** item **9**), and Sample Buffer (background control) to appropriate wells in triplicate.

4. In order to reach the maximal binding equilibrium, cover the wells with sealing film and incubate the strip wells overnight (16 ± 1 h) at 4 ºC.

5. Dilute the PAR polyclonal detecting antibody 1:250 fold in Antibody Diluent and incubate at 25°C one hour before use (Section V item 4). Bring the incubated stripwells to room temperature. Gently remove the plate sealer and wash strip wells 4 times with PBST (300 µl well). Ensure that all liquid is removed by tapping strip wells onto paper towels.

6. Add 50 µl per well of diluted PAR polyclonal detecting antibody (prepared in section **V** item **4**). Cover the wells with sealing film and incubate at 25 ºC for 2 hours.

7. Dilute the HRP conjugate 1:250 in Antibody Diluent and incubate at 25°C one hour before use (Section V item 5). Gently remove the plate sealer and wash strip wells 4 times with PBST (300 µl/well). Ensure that all the liquid is removed by tapping strip wells onto paper towels.

8. Add 50 µl per well of diluted Goat anti-Rabbit IgG-HRP conjugate (prepared in section **V** item **5**). Cover the wells with sealing film and incubate at 25°C for 1.0 hour. Place PeroxyGlow ™ A and B reagents at 25 ºC to pre-warm.

9. Gently remove the plate sealer and wash strip wells 4 times with PBST (300 µl/well). Ensure that all the liquid is removed by tapping strip wells onto paper towels.

10. Just before use, mix equal volumes of PeroxyGlow™ A and B together and add 100 µl per well. **Immediately** take chemiluminescent readings.

VIII. Data Interpretation

Calculate the net mean RLU (Relative Light Units) values of the PAR standards by subtracting the background (without PAR) from the RLU values. Plot the net mean RLU as a function of PAR values (pg/ml) as depicted in **Figure 1**.

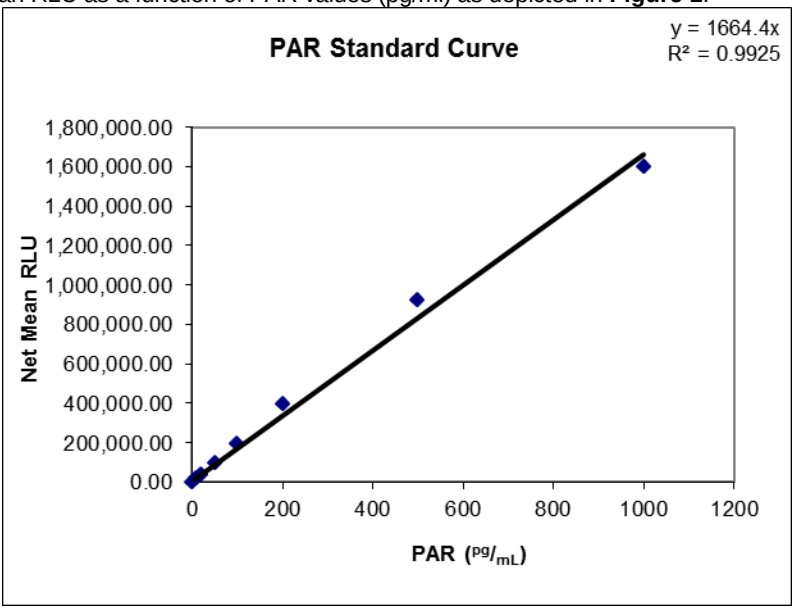

Figure 1. Typical PAR standard curve with trend line. The linear dynamic range is from 10 to 1000 pg/ml. The R^2 value for the linear curve fit (shown) was 0.9945.

Calculate the net RLU values of the Jurkat Cell Lysate Standards, PBMC or Xenograft extracts by subtracting the background from the RLU values. Determine the PAR levels in each sample using a standard curve (e.g. Figure 1). Use a linear regression line and set the y-intercept equal to zero.

IX. References

1. Virag, L., and Szabo, C. 2002. The therapeutic potential of Poly(ADP-Ribose) Polymerase inhibitors. *Pharmacological Reviews* **54**:375-429.
2. Thiemermann, 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.
3. Curtin NJ. 2005. PARP inhibitors for cancer therapy. *Expert Rev Mol Med* **7**:1-20.
4. Kinders JK, Hollingshead M, Khin S, Rubinstein L, Tomaszewski JE, Doroshow JH, Parchment RE, and the National Cancer Institute Phase 0 Clinical Trials Team. 2008. Preclinical Modeling of a Phase 0 Clinical Trial: Qualification of a Pharmacodynamic Assay of Poly (ADP-Ribose) Polymerase in Tumor Biopsies of Mouse Xenografts. *Clin Cancer Res* **14**:6877-85.

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 µM), 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.
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 and check the linearity by back-calculating pg/mL PAR per cell number or per microgram of 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 PBST between steps.
	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.5 and VII.7)

PROBLEM	CAUSE	SOLUTION
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
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 standards; or, 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 perfor-mance of the preincubation step

XI. Related Products Available From Trevigen

Kits:

Catalog #	Description	Size
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
4667-50-03	3-Aminobenzamide (200 mM)	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

Appendix A: Assay Validation

Assay Specificity

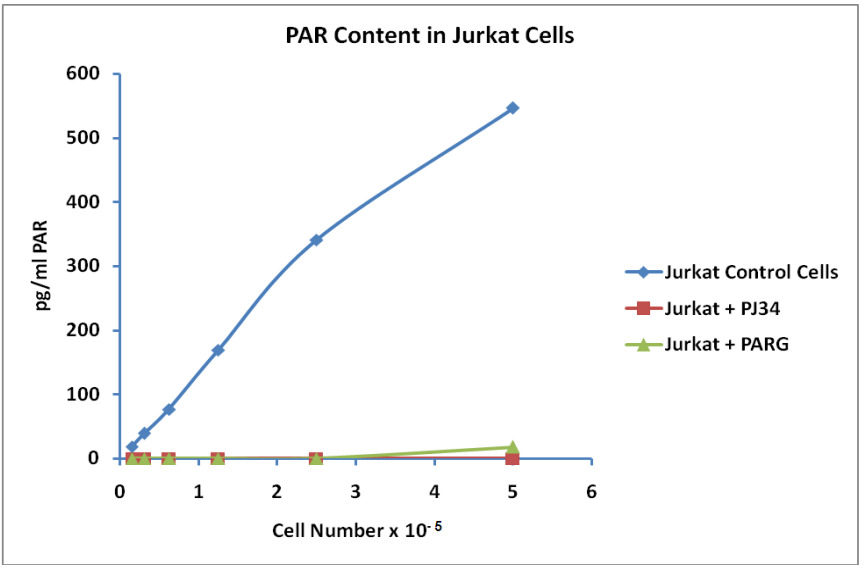

Figure 2. Assay specificity was established using Jurkat cells (ATCC, cat# CRL-2570) exposed either to PJ34, a potent PARP inhibitor, or to PARG, which digests PAR into subunits. Jurkat cells were exposed to 1 µM PJ34 (Calbiochem cat# 528150) for 1.5 hr and then harvested (PJ34 was included in the Cell Lysis Buffer), and extracts were prepared. PARG (10 ng, cat# 4680-096-01) was added to an aliquot of Jurkat cells during the lysis procedure and incubated at room temperature for 30 minutes prior to the addition of SDS. The lysates were then tested. Control Jurkat cells are untreated.

Assay Reproducibility

Validation of assay reproducibility was determined by obtaining peripheral blood mononuclear cells (PBMC) from three healthy donors. After isolation, PBMC were aliquoted, and frozen. PBMC lysates were prepared and assayed on three successive days. Variation is minimal indicating the assay is well suited to obtain baseline PAR levels as shown in figure 3. Included in the assays are Jurkat cell extracts containing high (807 ± 9), medium (182 ± 1.5), and low levels (31 ± 2.9) of PAR used as positive controls (figure 4).

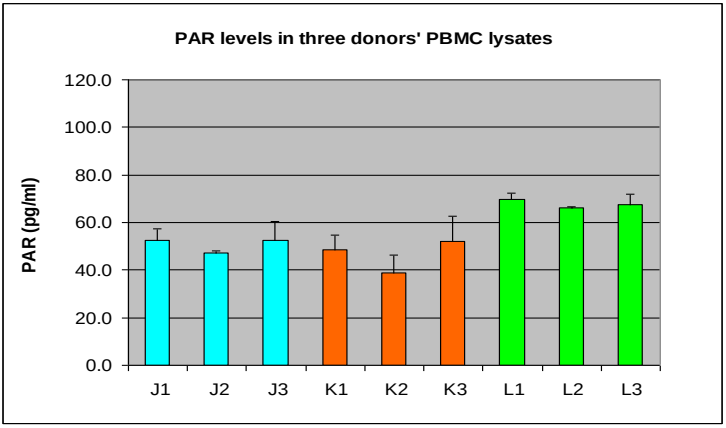

Figure 3. Baseline PAR levels in PBMCs from normal donors (J, K, and L) expressed in terms of pg/ml per 1X10^7 cells/ml. PBMC lysates were made on three days and each was assayed in triplicate. The means and standard deviations of each determination are shown.

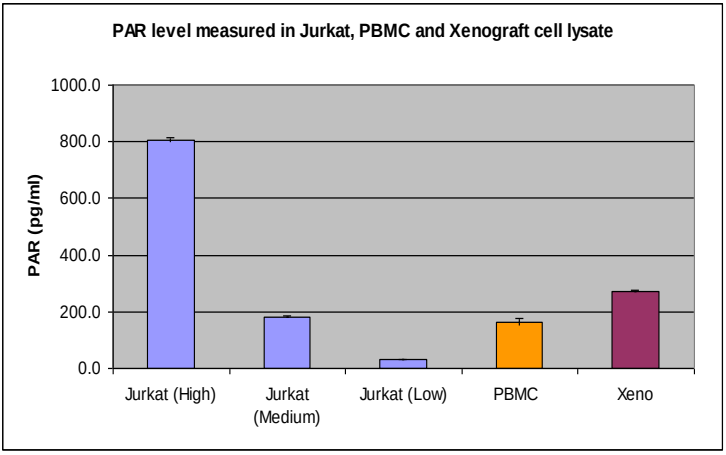

Figure 4. Jurkat Cell Lysate Standards, PBMC, and Xenograft PAR levels as measured using Trevigen's HT PARP in vivo Pharmacodynamic Assay II: PAR levels are also expressed in terms of pg per 1X10^7 extracted PBMC, or pg per 100 µg Xenograft extract.

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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