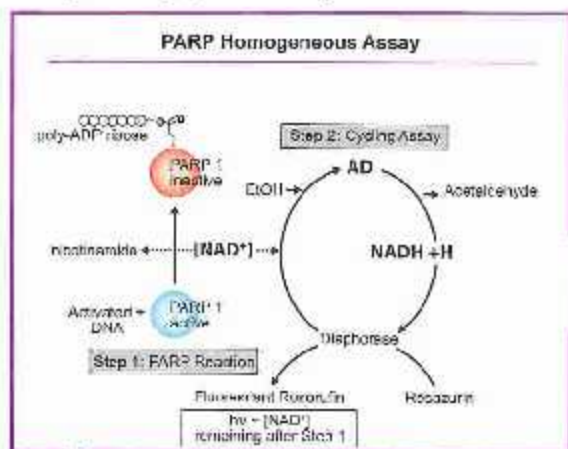


PARP/ PAR/ PARG  
PARP Assay Selection Guide

High Throughput Screening for PARP Inhibitors

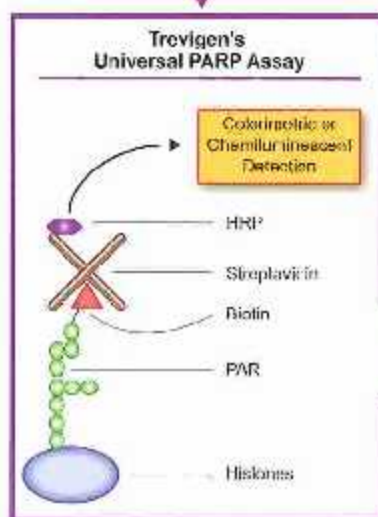


HT F Homogeneous PARP Inhibition Assay Kit # 4690-096-K

Verification of Lead Compounds or Limited Inhibitor Screening Campaigns. Determination of IC<sub>50</sub> Values

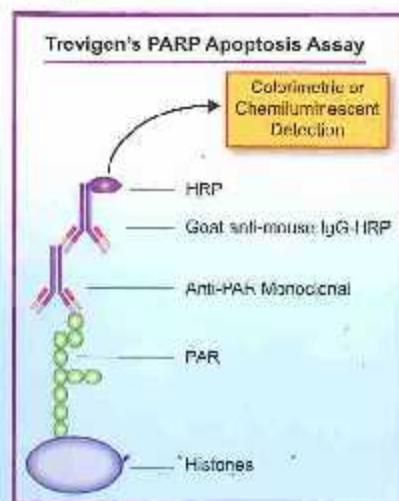
Determine PARP Activity in Cell Lysates

HT PARP/ Apoptosis Assay Kit # 4684-096-K Colorimetric # 4685-096-K Chemiluminescent

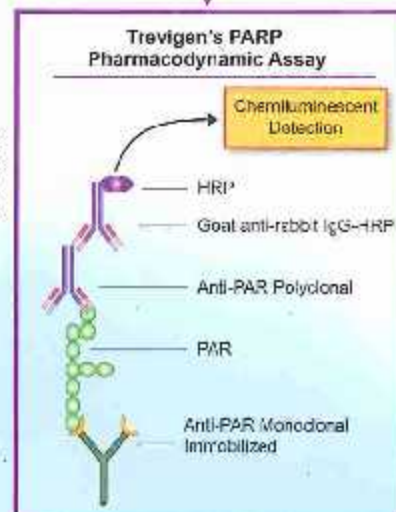


HT Universal PARP Assay Kits # 4677-096-K Colorimetric # 4676-096-K Chemiluminescent

Evaluation of Inhibitor Behavior *In Vivo* or in Cultured Cells



HT PARP *in vivo* Pharmacodynamic Assay II # 4520-096-K



*Trevigen Cell Assays (TCA), a division of Trevigen, Inc., was established in 2008 to conduct contract research for the pharmaceutical, biotechnology, government and academic segments of the medical research market. TCA specializes in designing and conducting assays for lead compounds and genotoxic screening based on DNA damage and repair as well as cancer cell behavior.*



## PARP/PARG Assays

In response to genotoxic stress Poly(ADP-ribose) polymerases (PARP 1 and PARP 2) are rapidly activated by DNA strand breaks. Once activated, NAD<sup>+</sup> is consumed for the synthesis of highly negatively charged polymers of ADP-ribose (PAR) on target nuclear proteins that include PARP itself 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<sup>+</sup> and in turn reduce the capacity of the cell to generate energy in the form of ATP resulting in cell death.

Trevigen offers kits that measure the in vivo and in vitro activities of PARP 1, PARP 2, and PARG. One hallmark of apoptosis is the caspase-mediated cleavage and inactivation of PARP 1. The PARP/Apoptosis Kit measures decreasing levels of PARP activity as cells move through the apoptotic pathway. The Homogeneous PARP assay is well suited for the large scale screening of compound libraries. Our Universal PARP Assay and PARG Assay are designed to analyze small numbers of inhibitors providing accurate IC<sub>50</sub> information. Once inhibitors of PARP have been identified using in vitro screening assays, their in vivo activity in cell extracts or peripheral blood mononuclear cells can be measured using Trevigen's PARP in vivo Pharmacodynamic Assay II.

Indicate kit(s) to be run using your samples. If you are unsure of the proper kit to use, please let us know what is to be measured (i.e. PARP<sup>1</sup>, PAR, etc.) and we can aid you in identifying the proper kit from the summary of assays available in the table below.

	Catalog No.	Application(s)	Detection Method	Test Sample	Sensitivity
<b>Universal PARP Assay Kits</b>					
• Colorimetric Detection	4677-096-K	PARP Inhibitor Screen	Biotin NAD <sup>+</sup> Histone ribosylation	PARP Inhibitors	0.01-1 Units PARP
• Chemiluminescent Detection	4676-096-K	IC <sub>50</sub> Determination			
<b>Homogeneous PARP Inhibition Assay</b>					
• Fluorescence Detection	4690-096-K	HI PARP Inhibitor Screen	NAD <sup>+</sup> Consumption PARP ribosylation	PARP Inhibitors	10% PARP Inhibition
<b>PARP/Apoptosis Assay Kits</b>					
• Colorimetric Detection	4684-096-K	PARP Inhibitor Screen	PAR Ab Histone ribosylation	PARP Inhibitors	0.1-10 mUnits PARP
• Chemiluminescent Detection	4685-096-K	In Lysates		Cell Lysates	500 cells
<b>PARG Assay Kits</b>					
• Colorimetric Detection	4663-096-K	PARG Inhibitor Screen	Biotin NAD <sup>+</sup> PAR degradation	PARG Inhibitors	250 pg PARG
• Chemiluminescent Detection	4662-096-K				
<b>Homogeneous PARG Inhibition Assay</b>					
• Chemiluminescent Detection	4691-096-K	PARG Inhibitor Screen in vitro	Luciferase, PAR degradation	PARG Inhibitors	10% PARG Inhibition
<b>PARP in vivo Pharmacodynamic Assay II</b>					
		See FORM TCA 2 for PDA II questionnaire.			
<b>Tankyrase I Assay</b>					
• Colorimetric Detection	4700-096-K	Tankyrase Inhibitor Screen	PAR Ab Histone ribosylation	Tankyrase Inhibitors	0.1-10 mUnits PARP
• Chemiluminescent Detection	4701-096-K				500 cells



FORM TCA 3 DATE

## PARP/PARG Assay

Getting started is easy. Fax the completed form to 301-560-4973; or email us at [info@trevigenceassays.com](mailto:info@trevigenceassays.com) or complete the form online at [www.trevigenceassays.com](http://www.trevigenceassays.com) with the information that we need in order to set up your screening service. We will require a purchase order and down payment before we begin work.

### Quotation Request Form - PARP/PARG Assay

First and Last Name

Email Address

Company

Street Address

City  State/Province

Zip/Postal Code  Country

Telephone Number  Fax Number

What type of assay/kit is required?  PARP - Cat. #   PARG - Cat. #   Tankyrase - Cat. #

Please refer to the summary of assays available on reverse of this page and check the appropriate assay writing in the catalog. If unsure of the proper kit to use, please let us know what is to be measured (i.e. PARP1, PAR, etc.) and we can aid you in identifying the proper kit for your needs.

#### General Questions

What is the desired reporting format?

How soon is the data required?

How many replicates are required?

Are there any other screening parameters or special conditions that you require?

#### Compound Screening

How many compounds and concentration range to be tested do you have to screen?

#### Compound Handling Instructions

What compound(s) are you screening?

If you are not providing the compound(s), where can they be purchased?

Is the compound toxic? If yes, are MSDS available?

What storage conditions are required?

What is the solubility of the compound?

#### If Lysates Are Being Investigated: (PARP/Apoptosis kits only)

Are you providing lysates or will treatment be done by Trevigen?

If treatment is being done by Trevigen, what type of cells are required?

Will you be providing the cells?

Do the cells require any special handling?

What type of damaging agent is required?

Upon receipt, a TCA senior scientist will contact you to go over the desired work and discuss options as appropriate. A proposal and cost estimate will then be prepared. The proposal will include the turnaround time and the agreed upon reporting format.

established in 2008 to conduct contract research for the pharmaceutical, biotechnology, government and academic segments of the medical research market. TCA specializes in designing and conducting assays for lead compounds and genotoxic screening based on DNA damage and repair as well as cancer cell behavior.



## HT PARP in vivo Pharmacodynamic Assay

### The PDA II kit is for RESEARCH USE ONLY

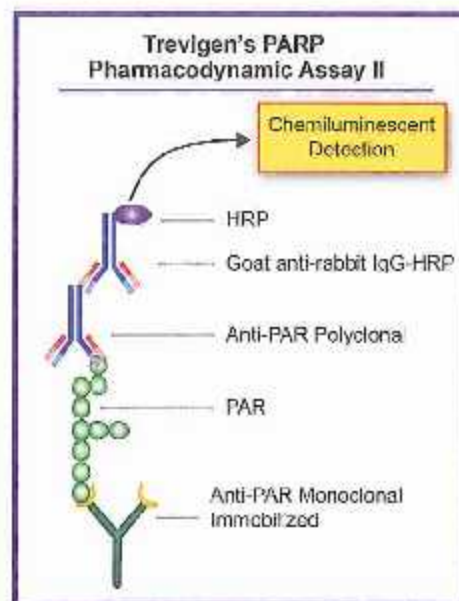
PARP catalyzes the NAD<sup>+</sup>-dependent addition of poly (ADP-ribose) (PAR) onto itself and adjacent nuclear proteins. This enzyme is a therapeutic target for BRCA1 and BRCA2 associated breast cancers. To address the need to monitor PARP activity among different individuals and within cells, Trevigen's improved and validated HT PARP *in vivo* Pharmacodynamic Assay II measures net PAR levels in tissue or cellular extracts and has been used to document differences in PAR levels among tumor lysates, organs and xenografts. The HT PARP *in vivo* Pharmacodynamic Assay II employs a 96 well plate, pre-coated with Trevigen's monoclonal PAR antibody as the capture agent, and PAR polyclonal rabbit antibody as the detecting agent.

### Assay Design

**Step 1:** Immobilized PAR mAb captures cellular PAR and PAR attached to proteins in prepared lysates.

**Step 2:** Binding of PAR polyclonal detecting Ab to capture PAR.

**Step 3:** Measure captured PAR via binding of goat anti-rabbit IgG-HRP with chemiluminescent detection.  
• Light output (Signal) correlates with the amount of cellular PAR.



	Catalog No.	Application(s)	Detection Method	Test Sample	Sensitivity
<b>PARP in vivo Pharmacodynamic Assay II</b>		PARP Inhibition in vivo, PAR Levels in Lysates	Chemiluminescent	PMBC Lysates	2-1000
• Chemiluminescent Detection	4520-096-K		ELISA	Tissue Lysates	pg/ml PAR

## HT PARP in vivo Pharmacodynamic Assay

Getting started is easy. Fax the completed form to 301-560-1973; or  
or complete the form online at [www.trevigencellassays.com](http://www.trevigencellassays.com) with the information that we need in  
order to set up your screening service. We will require a purchase order and down payment before  
we begin work.

### Quotation Request Form - PDA II Assay

First and Last Name \_\_\_\_\_

Email Address \_\_\_\_\_

Company \_\_\_\_\_

Street Address \_\_\_\_\_

City \_\_\_\_\_ State/Province \_\_\_\_\_

Zip/Postal Code \_\_\_\_\_ Country \_\_\_\_\_

Telephone Number \_\_\_\_\_ Fax Number \_\_\_\_\_

#### General Questions

What is the desired reporting format? \_\_\_\_\_ How soon is the data required? \_\_\_\_\_

How many samples will be tested? \_\_\_\_\_ How many replicates are required? \_\_\_\_\_

Are there any other screening parameters or special conditions that you require? \_\_\_\_\_

What type of samples are being tested? \_\_\_\_\_

#### If Clinical Research Samples:

Are the samples part of a clinical trial? \_\_\_\_\_

If yes, will coordination with different clinical sites be required? \_\_\_\_\_

Do you require training in sample preparation? \_\_\_\_\_

#### If Drug Discovery Samples:

Are you providing the samples or will treatment be done by Trevigen Cell Assays? \_\_\_\_\_

#### If Treatment Is Being Performed By Trevigen Cell Assays

How many compounds and concentration range to be tested do you have to screen? \_\_\_\_\_

How many time points? \_\_\_\_\_ What is the treatment duration? \_\_\_\_\_

What type of cells will be used? \_\_\_\_\_

Will you be providing the cells? \_\_\_\_\_

Do the cells require any special handling? \_\_\_\_\_

What type of damaging agent is required? \_\_\_\_\_

#### Compound Handling Instructions

What compound(s) are you screening? \_\_\_\_\_

Will you be providing the compound(s)? \_\_\_\_\_

If you are not providing the compound(s), where can they be purchased? \_\_\_\_\_

Is the compound toxic? If yes, are MSDS available? \_\_\_\_\_

What storage conditions are required? \_\_\_\_\_

What is the solubility of the compound? \_\_\_\_\_

Upon receipt, a TCA senior scientist will contact you to go over the desired work and discuss  
options as appropriate. A proposal and cost estimate will then be prepared. The proposal will  
include the turnaround time and the agreed upon reporting format.