Cultrex® 96 Well 3D Spheroid BME Cell Invasion Assay
Catalog# 3500-096-K • 96 samples

Kit components
• 10X Spheroid Formation ECM
• 96 Well Spheroid Formation Plate
• Invasion Matrix

Features:
• Complete kit; just add cells and media.
• Spheroid Formation ECM and Spheroid Formation Plates promote spheroid formation for many epithelial and mesenchymal tumor cell lines which were previously thought incompatible with this model.
• Invasion can be monitored and quantitated in real-time.
• Extended invasion time allows for interrogation of compounds requiring replication.
• No specialized equipment required. Images can be captured using a bright field microscope with camera, and analyzed using free ImageJ software (instructions provided).

The need for more complete and physiologically predictive cancer invasion models has driven the development of the 96 Well 3D Spheroid BME Cell Invasion Assay. There is growing evidence that tumor cell aggregates or spheroids are more representative of tumors in vivo, and they exhibit several physiological traits including similar morphology, the formation of cell-cell bonds, and gradients for nutrients, oxygen, pH, and catabolites due to limitations in diffusion through multicellular layers. Applying this model to a 3D culture invasion assay provides a more physiological approach for assessing tumor invasion and offers a visual component that can be quantitated through image analysis.

Figure 1. Illustration of protocol for the Cultrex 96 Well 3D Spheroid BME Cell Invasion Assay.

Figure 2. Invasion of MDA-MB-231 human breast cancer cells out of spheroids into the surrounding Invasion Matrix as a function of time.

Figure 3. Inhibition of MDA-MB-231 spheroid cell invasion by Sulforaphane in a dose-dependent manner after 4 days of invasion.
Cultrex® Cell Harvesting Kit (human/mouse)

Features:

• First standardized kit for isolating cells from 3D culture for biochemical analysis
• Non-enzymatic formulation preserves extracellular receptors and prevents subsequent degradation during processing.
• Overcomes the issue of extracting cells from a BME or Laminin I in 3D models – improvement on existing cell harvesting techniques
• Includes buffers for cell lysates and a G3PDH antibody for normalizing loading
• Ideal for Western Blotting
• May also be used for RNA and DNA analysis

Kit Contents:

10X Cell Harvesting Buffer, 100 ml
10X Cell Wash Buffer, 100 ml
Sample Buffer, 10 ml
10X Loading Buffer, 1 ml
Anti-h/m G3PDH, 20 ml

Figure 1. Morphology of MCF-10A (A,B) and MCF-7 cells (C,D) in traditional 2D culture and 3D BME culture, scale = 250 μm.

Figure 2. Harvesting cells from 3D Culture.

Figure 3. Evaluation of expression of RNA expression via RT-PCR (A) and protein expression via Western blotting (B) for GAPDH from MCF-10A and MCF-7 mammary epithelial cells isolated from either traditional 2D culture or 3D BME culture.
Cultrex® 3D Cell Proliferation Assays

Kit Contents:
- Extracellular Matrix Protein (BME)
- Collagen I, Laminin I
- Cell Proliferation Reagent, 5 ml
- 96 sample stripwell plates, 2/kit

Features:
- First standardized kits engineered for assessing the effects of compounds or genetic alteration on cell proliferation in 3D Culture
- Available with Cultrex® Basement Membrane Extract (BME), Laminin I, or Collagen I
- Provides protocols for tumorigenicity and cytotoxicity
- High Throughput 96 well strip well format
- One step colorimetric detection
- Quantitative Analysis
- This assay can determine EC50 Values (effective concentration) of between 1000 - 100,000 cells per well.

Recent studies indicate that the composition of the extracellular environment influences the cellular response to apoptosis inducing agents\(^1,2\), implicating a role for extracellular proteins in influencing both toxicity and drug resistance. As a result, this environment must be mimicked during the course of cell-based studies to provide the most accurate translation to animal models. The Cultrex® Cell Proliferation Assays were created in an effort to provide more physiologically relevant assessment when using cell models in the screening process for compounds that influence toxicity, cell survival, and tumorigenicity, new tumor formation. These assays offer a flexible, standardized, high-throughput format for quantitating the degree to which pharmacological compounds influence toxicity or tumorigenicity in an in vivo-like environment.

References

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Figure 1. The presence of 3D hydrogels has no significant effect on readout. Stripwells were coated with 35 µl of either Collagen I, Laminin I, or BME, and allowed to polymerize at 37°C. MDA-MB-231 cells were serially diluted and seeded in both coated and uncoated stripwells as indicated on the graph, and 15 µl of 3D Culture Cell Proliferation Reagent was added to each well. Absorbance at 450 nm was recorded after 2 hours.

Figure 2. Illustration of protocol for assessment of tumorigenicity in 3D culture.

Figure 3. Colorimetric readout for 96 sample stripwell plate.

Figure 4. Proliferation of MDA-MB-231 cells in different extracellular environments in the presence of 5% FBS. 3D Culture was conducted using the tumorigenicity protocol. Briefly, cells were seeded in the presence or absence of ECM proteins and treated with 2 hours. Cell cultures were incubated at 37°C 5% CO\(_2\) for 4 days. Then 15 µl of 3D Culture Cell Proliferation Reagent was added to each well, and absorbance at 450 nm was determined at 2 hours. Values were assessed as a percentage of untreated controls.
CultreCoat® BME 96 Well Cell Invasion Assays

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Kit components
• BME-coated chambers
• Assay Buffers
• Calcein-AM

Features:
• Three different coating densities
• Pre-coated inserts save time
• Pre-coated inserts improve reproducibility
• Complete kit format
• Coating process assures uniform layers of BME plate to plate, and lot to lot, to minimize variability
• May be adapted for high throughput screening (robotic)
• Direct detection in receiver plate
• Black receiver plate minimizes crosstalk and background

CultreCoat 96 Well Cell Invasion Assays were created in an effort to accelerate the screening process for compounds and pathways that influence cell migration through extracellular matrices. These assays offer a flexible, standardized, high throughput format for quantitating the degree to which invasive cells penetrate a barrier consisting of basement membrane components in vitro in response to chemoattractants and/or inhibiting compounds.

We appreciate the fact that all cell lines do not exhibit the same invasive capacity, so the CultreCoat 96 Well BME Cell Invasion Assay has been adapted to three different matrix coating densities to provide researchers with a standardized approach for generating optimal results.
The vascular endothelium is the thin monolayer of cells that lines blood vessels and provides a network for the exchange of biological materials such as gases, nutrients, and metabolic waste throughout the body. The endothelium exhibits a selective barrier function between the vessel lumen and the surrounding tissues which controls this exchange. There are many vasoactive cytokines and growth factors that function in regulating the degree of vascular permeability, such as interleukin alpha and beta, TNF-alpha, IFN gamma, hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF). There are also several systemic diseases associated with disruption of vascular permeability, such as cancer, diabetes, heart disease, stroke, hypertension, and arthritis. These assays offer a flexible, standardized, high-throughput format for quantitating the degree to which genes or compounds can influence the maintenance of endothelial cell-to-cell adhesion.

References
CometAssay® Electrophoresis System II

Features and Benefits
• 21 cm in length
• Run at 1V/cm, ~300 mA for 30-40 minutes
  • Use with most laboratory power supplies
• No well to well variation
  • Overlay to maintain 0.25cm buffer height
  • 1L of buffer, 150-300 mM NaOH pH >13
• Platform accommodates 1 row of slides
  • Maintain slide position
  • Use 2, 20 and 96 well slide formats
    • 20, 100 or 288 samples in 1 run
  • Easy removal and placement of slides
• Maintain buffer temperature ~ 4°C
  • Cooling chamber

A. Control Cells

B. Minimal Well to Well Variation

C. Buffer Height

D. Slide Formats
Alkaline Overview
Cells mixed with LMAgarose
Treat with Lysis Solution
Equilibrate/Electrophoresis
200 mM NaOH pH >13
Fix Samples
70% EtOH
Stain and Visualize
Droplet Shape

Neutral Overview
Cells mixed with LMAgarose
Treat with Lysis Solution
Equilibrate/Electrophoresis
Na Acetate pH 9 Buffer
Fix Samples
1M NH₄Ac/90% EtOH
70% EtOH
Stain and Visualize
Elongated Shape

5' → 3' → 5' → 3' → 5' → 3' → 5' → 3' → 5' → 3' → 5' → 3' → 5' → 3' → 5'
Strand denaturation
Alkaline Comet Assay®

- DNA is denatured during electrophoresis
- Dose response
  - Single- or double-strand damaging agents

**Bleomycin**

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**Hydrogen Peroxide**

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H₂O₂
Bleomycin
Neutral CometAssay®

- DNA is not denatured during electrophoresis
- Dose response
  - Double-strand damaging agents only

**Bleomycin**

- Healthy
- T2
- T3

**Hydrogen Peroxide**

- Healthy
- T2
- T3

Tail Moment

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**96-Well CometChip™ System**

**Step 1: Cell Loading**
- Add 10,000 cells per well
- Cover wells with overlay
- Place in TC incubator, 30 min
- Aspirate excess cells
  ~ 240 micropores for each well

**Step 2: Cell Treatment**
- Add DNA damaging agent to designated wells
- Cover wells with overlay
- Place in tissue culture incubator

**Step 3: Alkaline CometAssay**
- Remove CometChip™ from Macroformer
- PBS rinse, overlay w/ LMAgarose
- Immerse in Lysis Solution (1-24 hr, 4°C)
- Perform Alkaline Unwinding (1 hr, 4°C)
- Perform Alkaline Electrophoresis

**Step 4: Fluorescent Detection**
- Analyze wet gels
  ~50-75 comets per 4X image

**Macroformer**
- Creates 96 separate wells by inserting CometChip™ into magnetically sealable cassette

**CometChip™**
- Agarose array
- 30 µ pores

**Bright field image: Cell loading into 30 µ pores**

**Etoposide Treatment**
- Multiple treatments on single slide

**Untreated**

**5 µM Etoposide**

- No overlapping comets
- No leakage between columns (wells)
**HT γ-H2AX Pharmacodynamic Assay - Detecting DNA Double Strand Breaks (Cat# 4418-096-K)**

**Features and Applications**

**HT γ-H2AX Pharmacodynamic Assay**
- Pre-coated γ-H2AX antibody plates
- Broad linear dynamic range from 10 to 800 pM
- High signal to noise ratio
- Highly sensitive to 5 pM
- Highly reproducible
- High throughput ELISA 96 test size

**Applications**
- Quantitation of γ-H2AX in human PBMC (peripheral blood mononuclear cells), tissue and culture cells.

**Kit Components:**
- γ-H2AX Standard, 1 µM
- anti-γ-H2AX Pre-coated 96-well plate
- H2AX IgM antibody
- Goat anti Mouse IgM-HRP
- Jurkat Lysate Control

**Figure 1:** Schematic chart of γ-H2AX ELISA Assay working mechanism.

**Figure 2:** γ-H2AX ELISA Assay Standard Curve. Dynamic range from 10 pM to 800 pM with sensitivity at 5 pM of γ-H2AX.

**Figure 3:** γ-H2AX values from Jurkat Cells treated with Bleomycin. Jurkat cells treated at 5x10⁶ cells/ml with 0.02 mg/ml, 0.2 mg/ml and 1.0 mg/ml of Bleomycin respectively.

**Figure 4:** Comparison of the γ-H2AX ELISA Assay and Western Blot to detect γ-H2AX levels. Jurkat cells were treated with different concentrations of Etoposide (1µM, 5µM, 10µM, 25 µM and 50 M) for 2hr at 37 ºC in RPMI+10%FBS. Western Blot was tested by using Trevigen anti γ-H2AX (Cat# 4418-APC-100).

**Figure 5:** Comparison of the γ-H2AX ELISA Assay and Western Blot to detect γ-H2AX levels. Jurkat cell lines were treated with 30 µM of Etoposide for different incubation time (0.5 hr, 1 hr, 2hr, 3 hr and 4 hr) at 37 ºC in RPMI+10%FBS. Western Blot was tested by using Trevigen anti g-H2AX (Cat# 4418-APC-100).
HT BPDE ELISA Assay in detection of PAH exposures

Introduction

Benzo[a]pyrene (BP) is a widespread environmental polycyclic aromatic hydrocarbon (PAH) pollutants found in cigarette smoke, charred food, exhaust from internal combustion engines and coal-burning factories [1]. A highly reactive diol epoxide derivative, BPDE, is the major electrophilic, mutagenic, and carcinogenic metabolite involved in covalent binding to DNA and proteins (Figure 1) [2]. Bound BPDE adducts serve as a biomarker for exposure as well as the body’s metabolic response to this environmental mutagen associated with the increased risk of lung and breast cancers [3-6]. Human BPDE-HSA adducts have inherent advantages over DNA adducts as measures of exposure for epidemiologic studies [7]. To address this need Trevigen offers a validated HT BPDE competitive ELISA for the detection of BPDE adducts in plasma, serum and cell culture supernatant samples and also a highly sensitive BPDE competitive ELISA for the detection of BPDE adducts in the sample.

Methods

Trevigen’s BPDE monoclonal antibody allows detection and quantitation of BPDE adducts in serum, plasma and cell culture samples. In competitive ELISA, BPDE adduct in sample or standard competes with Biotinylated BPDE-HSA for a limited antibody binding sites on the plates. Addition of chemiluminescence substrate results in inverse signal proportional to the concentration of BPDE adducts in the sample. In sandwich ELISA, the assay employs a pre-coated anti-BPDE monoclonal capture antibody, a biotinylated anti-human HSA polyclonal detecting antibody, and a chemiluminescence detection substrate, generating a highly sensitive and throughput assay flexible for the experimental design. Important features of the assay include: 1) chemiluminescent, non-radioactive format; 2) high throughput 96 strip wells; 3) dynamic range from 0.63 nM to 40 nM BPDE-HSA; and, 4) high sensitivity (LLD of 0.5 nM BPDE-HSA).

Results

BPDE standard curve generated by competitive ELISA assay is shown in Figure 3. BPDE adduct levels in health donor plasma were analyzed and results are shown in Figure 4.

Conclusions

Trevigen offers validated HT BPDE ELISA assays to detect BPDE-adduct and human BPDE-HSA levels commonly present in serum, plasma and cell culture supernatant upon PAH exposures. This assay has sensitivity and specificity equal to those reported previously (7) and can be applied in the monitoring of large number of samples in clinical and research studies.

References

HT 8-oxo-dG ELISA Assay II - Tool to study DNA damage
(Cat# 4380-096-K, 4380-192-K)

A validated HT 8-oxo-dG ELISA kit for the detection and quantitation of 8-oxo-dG in DNA, plasma, serum, urine and saliva samples.

Figure 1. Typical 8-oxo-dG competitive standard curve

Figure 2. Formation of 8-oxo-dG in Calf Thymus DNA by CrCl3/H2O2

Figure 3. Assay Validations of the 8-oxo-dG levels in Human Plasma Samples

Figure 4. Assay Validations of the 8-oxo-dG levels in Human Urine Samples

Table 1. Comparison of ELISA and LC/MS 8-oxo-dG in Calf Thymus DNA

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Features:
- Colorimetric, non-radioactive format; high throughput 96 strip wells;
- Dynamic range from 3.13 nM to 200 nM (0.89 ng/ml to 56.7 ng/ml); sensitivity at 2 nM (0.57 ng/ml) 8-oxo-dG.

Table 1. Comparison of ELISA and LC/MS 8-oxo-dG in Calf Thymus DNA

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<tr>
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<td>732.0</td>
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<td>3</td>
<td>828.0</td>
<td>351.3</td>
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<tr>
<td>Mean</td>
<td>789 ± 50.5</td>
<td>351.3 ± 3.2</td>
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8-OHdG Standard Curve:

\[
y = 0.8483x^2 - 3.7363x + 4.2899
\]

\[R^2 = 0.9994\]
Tools to Study Genomic Instability

Features and Benefits
1 million cells per vial.
• Target genes knockdown between 63%-98%.
• Lentivirus transduced LN428 cells.

Applications
• Analysis of DNA repair deficiency.
• Study Genomic Instability

Validation of knockdown cell lines

Enzymatic assay for MPG activity

Lane 1: Buffer alone
Lane 2: Control extract
Lane 3: MPG knockdown extract

Western Blot of MPG protein

Lane 1: Molecular weight markers
Lane 2: Scramble RNA Control
Lane 3: MPG knockdown extract
Lane 4: Control cell line

BRCA1 knockdown cell line is sensitive to PARP 1 inhibitor

Summary of 3 experiments

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Apoptosis and Related Products

Early Apoptosis – Cell Surface Changes
- Live Cell Assay
- Annexin V binding to PS exposed on outer cell membrane
- Flow Cytometry (Fluorescence)
  - Double-labeling to measure membrane integrity (PI)
- In situ labeling (Fluorescence or Colorimetric Microscopy)
- 100 or 250 tests
- Assay Time ~1 hr

Mid to Late Apoptosis – Fragmented DNA
- Identify apoptosis in variety of tissue types
- In situ Labeling (Fluorescence or Colorimetric Microscopy)
- Tissue specific optimized reagents (permeabilization and DNA labeling)
- 30 tests
- Assay Time ~3 hrs

Early to Mid Apoptosis – ∆ mitochondrial membrane potential
- Live cell assay
- Flow Cytometry or Fluorescence Microscopy
- Easy uptake of dye by cells
  - Healthy Cells – red fluorescence
  - Apoptotic Cells – green fluorescence
- 100 tests
- Assay Time ~20 min

Mid to Late Apoptosis – Enzymatic attachment of biotinylated dNTPs
- Apoptosis-specific nuclease cuts the DNA into fragments.
- Bind anti-biotin antibody (or Strep-HRP)
- Fluorescent/Colorimetric Detection

Catalog # | Related Products | Size
--- | --- | ---
4668-100-01 | Human Recombinant PARP Enzyme HSA | 1,000 units
4335-AMC-050 | PAR Polymer Affinity Purified Monoclonal Ab | 50 μl
4335-MC-100 | PAR Polymer Monoclonal Ab | 100 μl
4336-BPC-100 | PAR Polymer Polyclonal Ab | 100 μl
4338-MC-50 | PARP Monoclonal Ab | 50 μg
4500-10-P | PARP Treated Protein Control for Western | 10 μl
4677-096-P | Histone Coated Strip Wells (colorimetric) | 96 well
4678-096-P | Histone Coated Strip Wells (chemiluminescent) | 96 well
4850-20-ET | Apoptotic DNA Laddering Kit (Eth Br) | 20 tests
4890-025-K | MTT Cell Proliferation Assay | 2500 tests
4891-025-K | XTT Cell Proliferation Assay | 2500 tests
4411-PC-100 | Phosphorylated H2AX Polyclonal Ab | 100 μl
PARP Inhibitor Screening

Inhibitor Behavior \textit{in vivo}

Tools for PARP and PAR Analysis

Determine PARP Activity in Extracts

Trevigen’s PARP Homogenous Assay

Trevigen’s Universal PARP Assay

Trevigen’s PARP Apoptosis Assay

Trevigen’s PARP Pharmacodynamic Assay

Colorimetric or Chemiluminescent Detection

Histones
HT PARP in vivo Pharmacodynamic Assay
Cat# 4520-096-K

Features and Benefits
PARP in vivo Pharmacodynamic Assay
- Pre-coated capture antibody plates
- High signal to noise ratio
- Broad linear dynamic range to 1000 pg/ml
- Highly sensitive – detects 2 pg/ml PAR
- Reduced inter-assay variability
- Validated assay that measures drug action on PARP in both in vivo and in vitro settings
- 96 test size

Applications
- Quantitation of PAR in peripheral blood mononuclear cells, tissue culture cells, and tumor lysates from different tissues, organs and xenografts
- Monitoring the efficacy of PARP inhibitors on PAR formation in vivo
- Verifying observations of enhanced cancer cell cytotoxicity arising from PARP inhibitor/anticancer drug combination therapy
- Facilitating development of PARP and PARG targeted therapeutics

Kit Components:
- PAR Standard, 25 pg/µl
- Pre-coated 96-well plate
- PAR polyclonal antibody
- Goat anti Rabbit IgG-HRP
- DNase I, 2 units/µl
- 100X Magnesium Cation
- 20% (w/v) SDS
- Antibody Diluent
- Sample Buffer
- Jurakt Lysate Control (H,M,L)
- PeroxyGlow A & B substrate
- Cell Lysis Reagent

PAR Standard Curve - Validation 1-3

Validation 1-3 PAR levels in Jurkat lysates

Chemiluminescent Detection

Jurakt Lysate Control
PAR
PAR Polyclonal
Anti-PAR Monoclonal
Immobilized

PAR Levels from 3 donors from cells lysed on 3 different days

Lysates prepared from 3 donors on 3 different days

PAR (pg/ml)

Control (High)
Control (Medium)
Control (Low)
HT Fluorescent Screening Assay for PARP Inhibitors

Features:
- Homogeneous PARP Activity Assay
  - No wash steps
  - Highly sensitive fluorescent assay
  - Rapid - Assay takes ~1 hr
- Ideal for screening compounds which inhibit PARP activity
  - Inhibitors identified by increase in Fluorescent Signal
  - Detect as little as 10% inhibition of PARP Activity
- Determination of IC$_{50}$ values for PARP Inhibitors
- 96 test size

PARP Inhibition Assay

Step 1: PARP reaction requiring NAD$^+$ is performed.
  - As PAR polymer is synthesized NAD$^+$ is consumed from the reaction mixture.
  - PARP inhibitors prevent NAD$^+$ consumption.

Step 2: Cycling Assay is performed to quantify NAD$^+$ remaining from Step 1.
  - Each time NAD$^+$ cycles, a molecule of highly fluorescent resorufin is generated.
  - PARP inhibitors are identified by an increase in fluorescent Signal.

A. NAD Standards
- An NAD standard curve is performed with each experiment.
  - Standard curve used to determine IC$_{50}$ values for PARP inhibitors.
  - Detect as low as 10 nM NAD remaining in the PARP reaction.

B. Inhibition Assay
- In the absence of PARP activity maximal signal is observed.
  - Minimal signal is observed when PARP activity is present.
  - PJ34 show significant inhibition of PARP activity.

<table>
<thead>
<tr>
<th>PARP Inhibitors</th>
<th>Observed Ki$^*$</th>
<th>Published Ki</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-aminobenzamide</td>
<td>51 ± 10 nM</td>
<td>33 µM</td>
</tr>
<tr>
<td>4-amino1,8-naphthalimide</td>
<td>23 ± 7 nM</td>
<td>153-180 nM</td>
</tr>
<tr>
<td>6(5H)-phenanthridinone</td>
<td>408 ± 130 nM</td>
<td>305 nM</td>
</tr>
<tr>
<td>Benzamide</td>
<td>21 ± 5 µM</td>
<td>1-22 µM</td>
</tr>
<tr>
<td>PJ34</td>
<td>100± 55 nM</td>
<td>20 nM</td>
</tr>
<tr>
<td>EB47</td>
<td>25 ± 6 nM</td>
<td>45 nM</td>
</tr>
</tbody>
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Kit Components:
- 10X Cycling Enzymes
- 2 µM NAD
- PARP HSA Enzyme
- Activated DNA
- 10X Buffer
- Stop Solution
- 96-well Black Microplate

Cat#: 4690-096-K

Components:
- 10X Cycling Enzymes
- 2 µM NAD
- PARP HSA Enzyme
- Activated DNA
- 10X Buffer
- Stop Solution
- 96-well Black Microplate

Relative IC$_{50}$ values for known PARP inhibitors
**HT PARP/Apoptosis Assays**

**Features and Benefits**

**PARP/Apoptosis Assay**
- Colorimetric or Chemiluminescent readout
- 96 well format
- Highly sensitive – detects 0.1 mU PARP ~ 500 cells
- Dynamic range between 0.1 to 10 mU PARP
- Requires 10-100 ng extract for detection
- Assay Time ~2.5 hrs

**Applications**
- Measure activity in cell lines, primary or tumor cells, PBLs
- Measure activity before and after apoptosis

**Assay Design**

**Step 1:** Ribosylation of histones by PARP.
- PAR polymer synthesized onto histones immobilized in wells of 96 well plate using purified PARP or cell extracts
- PARP inhibitors and caspase 3 cleavage prevent synthesis of PAR polymer.

**Step 2:** Detection to measure incorporation of PAR attached to histones via PARP monoclonal antibody.
- Color/light output (Signal) is proportional to PARP activity.
- Monitor apoptosis by a decrease in PARP Signal.

**Kit Components**
- PARP-HSA and Buffer
- 10X PARP Cocktail
- Activated DNA
- NAD
- Histone-Coated Strip Wells
- PAR mAb and Diluent
- HRP Conjugate
- Detection Reagents
- Etoposide

**Colorimetric Readout**

**Standard Curve**

**Loss of PARP activity correlates with PARP cleavage during apoptosis.**

Western blot of a time course of cells treated with 50 µM etoposide for indicated times.

**PARP Activity in Cells Exposed to 50 µM Etoposide (apoptosis inducer).**

HT PARP/Apoptosis Assay
PARP activity decreases as a function of time. Each point represents the mean value from triplicate determinations and each reading represents the activity in 5,000 cells.

Cat#: 4684-096-K (colorimetric) 4685-096-K (chemiluminescent)
**Features and Benefits**

**PARP Activity Assay**
- Colorimetric or Chemiluminescent readout
- 96 well format
- Highly sensitive – detects 10 mU PARP/well (chemi)
- Assay Time ~5 hrs
- Available with histone-coated plate or histone reagent

**Applications**
- Measure PARP Activity in cell and tissue extracts
- Identify inhibitors and activators of PARP activity
- Determination of IC$\text{}_{50}$ values for PARP Inhibitors

**Assay Design**

**Step 1: Ribosylation of histones by PARP.**
- Biotinylated-PAR polymer synthesized onto histones immobilized in wells of 96 well plate using purified PARP or cell/tissue extracts.
- PARP inhibitors prevent synthesis of biotinylated-PAR polymer.

**Step 2: Detection to measure incorporation of biotinylated PAR onto histones via Strep-HP.**
- Color/light output (Signal) is proportional to PARP activity.
- PARP inhibitors are identified by a decrease in Signal.

**Kit Components:**
- PARP-HSA
- PARP Buffer and Cocktail
- Activated DNA
- Histone-Coated Strip Wells

**Cat#: 4671-096-K (colorimetric) 4676-096-K (chemiluminescent)**

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HT Luminescence Screening Assay for PARG Inhibitors

**Features:**
- Homogeneous PARG Inhibitor Assay
  - No wash steps
  - Highly sensitive luminescence assay
  - Rapid, one step - Assay takes ~30 minutes
- Ideal for screening compounds which inhibit PARG activity
  - Inhibitors identified by decrease in luminescence signal
  - Detect as little as 10% inhibition of PARG Activity
- Determination of IC$_{50}$ values for PARG Inhibitors
- 96 test size

**One Step PARG Inhibition Assay**
As PAR polymer is metabolized into ADP-Ribose it is converted to AMP which enters an enzymatic cycling reaction resulting in a quantitative light output that is stable over 30 minutes.
- ATP converted from AMP is consumed by luciferase in a light generating reaction, regenerating AMP.
- PARG inhibitors prevent PAR Polymer metabolism with a subsequent decrease in relative light output.

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**A. PARG Standards**
- A PARG standard curve is performed with each experiment.
- The standard curve is used to determine IC$_{50}$ values for PARG inhibitors.

**B. Inhibition Assay**
- In the absence of PARG activity minimal signal is observed.
- Maximal signal is observed when PARG activity is present.
- DEA inhibitor shows significant inhibition of PARG activity.

**Relative IC$_{50}$ value for known PARG inhibitor**

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<td>DEA</td>
<td>30 ± 10 µM</td>
<td>7 µM</td>
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**HT PARG Assay Kits**

**Features and Benefits**

**PARG Activity Assay**
- Colorimetric or Chemiluminescent readout
- 96 well format
- Highly sensitive – detects 50 pg PARG per well
- Assay Time ~6 hrs

**Applications**
- Measure PARG Activity in cell and tissue extracts
- Identify inhibitors and activators of PARG activity
- Determination of IC₅₀ values for PARG Inhibitors

**Assay Design**

**Step 1: Ribosylation of histones by PARP.**
- Biotinylated-PAR polymer synthesized onto histones immobilized in wells of 96 well plate using purified PARP.

**Step 2: PARG degrades PAR.**
- Biotinylated-PAR attached to histones is hydrolyzed by PARG.
- PARG has both an endo and exoglycosidase activity.

**Step 3: Detection to measure remaining biotinylated PAR attached to histones via Strep-HRP.**
- Decrease in Color/light output (Signal) proportional to PARG activity.
- PARG inhibitors are identified by an increase in Signal.

**Comparator to Standard Assay**

The disappearance of PAR is ~ 2 fold greater in the colorimetric assay than standard radiolabeling assay. All PAR (ADR-ribose and PAR chains) is detected in the colorimetric assay but not in the radiolabeling assay since short PAR chains won’t migrate with ADP-ribose using TLC.

Dr. Myron Jacobson, U of AZ

Cat#: 4682-096-K (colorimetric) 4682-096-K (chemiluminescent)