• TREVIGEN SCIENTISTS HAVE 150 YEARS OF COMBINED SCIENTIFIC EXPERIENCE •

• TREVIGEN IS THE RECIPIENT OF SEVERAL SBIR GRANTS FROM THE NIH FOR ANALYSIS OF DNA DAMAGE TECHNOLOGY

TCA

Trevigen Cell Assays

Custom Contract Services

TREVIGEN®

• TREVIGEN EMPLOYS EXPERTS IN THE AREAS OF APOPTOSIS, DNA DAMAGE AND CANCER CELL BEHAVIOR

• TREVIGEN PRODUCTS HAVE BEEN CITED IN THOUSANDS OF PEER-REVIEWED ARTICLES •

**Welcome to Trevigen Cell Assays (TCA)**, a division of Trevigen, Inc., it is a boutique CRO (Contract Research Organization) specializing in designing and conducting assays for lead compounds and genotoxic screening based on DNA damage and repair as well as cancer cell behavior. It was established in 2008 to conduct contract research for the pharmaceutical, biotechnology, government, and academic segments of the medical research markets. Since its inception, TCA has served many sectors of the pharmaceutical and academic research market and has had many repeat customers.


Michael T. Elliot,
President, Trevigen Inc.

"We built Trevigen on the premise of providing researchers with cutting edge tools that fulfill unmet needs and solve problems previously unaddressed. Our team has over 150 years of combined experience in serving the scientific community. The aim of Trevigen Cell Assays is to provide contract services for assays developed by Trevigen that are technically challenging, yet vital to furthering our customer's research. You can trust that my team understands the need for confidentiality and intellectual property protection."

## About Trevigen

Trevigen, Inc. is dedicated to serve the research community with the highest quality products for studies involving the characterization of cancer cell behavior, apoptosis, DNA damage, and genomic instability. Trevigen is the recipient of several SBIR grants from the National Institutes of Health, concerning technology development for the analysis of DNA damage. Trevigen brand products are manufactured to the highest standards of quality and backed by expert technical support. The service division of Trevigen (Trevigen Cell Assays) is an integral part of a mission of providing our customer a full-service option.

Jay George, Chief Scientific Officer

"As the Chief Scientific Officer of Trevigen, I strive to make sure our assays perform in the manner necessary to further your research, whether it be validating unique sample types, achieving greater sensitivity or increasing the dynamic range of the assay. Each team member plays an integral part in ensuring that you the customer are completely satisfied with both our products and our services."

Irina Arnaoutova, Senior Scientist

"As a senior scientist for Trevigen, I am continually striving to develop new and innovative products that will benefit our customer's research efforts. I split my time between bench work and perusing recent scientific literature. My goal is to assist in identifying and developing appropriate tools for your research. I will then implement our findings to ensure the success of your project."

## TCA Services Process

**1** Project initiation

**2** A Project Manager and a Senior Scientist is assigned to each project

**3** A detailed protocol and schedule is prepared and approved by you before the work begins

**4** Observations, comments, and updates are sent by e-mail during the study

**5** A final report and raw data with graphical analysis is provided

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TCA2012

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## TCA Services Available

| | Assay | Applications |
|---|---|---|
| **Comet Assay** | Rapid analysis of DNA fragmentation associated with DNA damage | • Detect and quanitate DNA damage<br>• Follow DNA repair |
| **PARP Assay** | Measurement of PARP1 mediated poly (ADP-ribose) ribosylation of histone proteins | • Assay inhibitors and activators of PARP activity<br>• Determination of $IC_{50}$ values for PARP inhibitors |
| **PARG Assay** | Measure the loss of PAR from histones | • Identify inhibitors and activators of PARG activity<br>• Determination of $IC_{50}$ values for PARG inhibitors |
| **Pharmacodynamic PARP Assay II** | Measurement of net PAR levels in cellular extracts and tumor lysates | • Quantification of PAR in peripheral blood mononuclear cells, tissue culture cells, and tumor lysates from different tissues, organs & xenografts<br>• Monitoring the efficacy of PARP inhibitors on PAR formation in vivo<br>• Facilitating development of PARP and PARG targeted therapeutics |
| **Cell Invasion Assay** | Analysis of responses to chemokines, toxins, drugs and other analytes of interest | Screening for compounds that influence cellular invasion through:<br>• Extracellular matrices • Tumor cell metastasis<br>• Angiogenesis |
| **Tankyrase 1 Assay** | Identification of Tankyrase 1 (PARP5A) inhibitors | Screening for compounds that inhibit Tankyrase 1 (PARP5A) and identification of $IC_{50}$ values |
| **Synthetic Lethal Screening** | Identification of compounds that inhibit cell growth as a result of dysfunctional DNA repair gene | Identification of synthetic lethal compounds |
| **Tube Formation** | Identification of angiogenesis inhibitors/promoters | Screening of compounds that inhibit or promote angiogenesis |

**FORM TCA 1 DATE** _____

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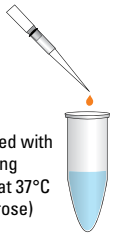
# Comet Assay Service

Trevigen is the sole provider of a standardized CometAssay system for the direct detection of DNA Damage. The CometAssay is a single cell gel electrophoresis assay which can be used to quantitate DNA Damage from in vivo or in vitro samples. The CometAssay measures double strand breaks (DSBs), single strand breaks (SSBs), alkali labile sites, oxidative DNA base damage, DNA-DNA/DNA-protein/DNA-Drug crosslinking and DNA repair. At your request, the scientists at TCA will collaborate with you to design screening studies employing the CometAssay tailored for your specific needs.

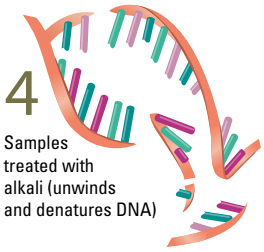
## Assay Procedure



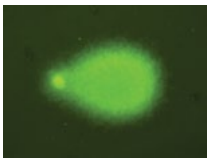
1 Cells mixed with low melting agarose at 37°C (LM Agarose)


2 Immobilize cells on CometSlide™

3 Treat cells with Lysis Solution (removes membranes and histones from the DNA)



4 Samples treated with alkali (unwinds and denatures DNA)



5 Samples stained with intercalating dye and visualized by epifluorescence microscopy following alkaline electrophoresis, which reveals DNA breaks

## Advantages

- TCA uses a unique platform of standardized comet assay kits, slides, control cells, CometAssay ES electrophoresis system (patent applied for) and state of the art image analysis to assure consistency of results with successive experiments or screening studies.
- The technical team at TCA developed the reagents, slides, control cells and electrophoresis system, and is unmatched in familiarity and skill with the comet assay.
- TCA has already successfully executed multiple comet assay contracts and thus has a proven track record of high performance.
- Confidentiality is assured. TCA is ready to execute Non-Disclosure Agreements as required.

## Also Available

TCA has expanded its services to offer study design and compound screening services for:

- PARP in vivo Pharmacodynamic Assay II (FORM TCA 2)
- PARP/PARG Assays (FORM TCA 3)
- Cell Invasion/Migration (FORM TCA 4)

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**FORM TCA 1 DATE** _____

# Comet Assay

*Getting started is easy. Fax the completed form to 301-560-4973; or email us at TCA@trevigen.com; or complete the form online at www.trevigencellassays.com with the information that we need in order to set up your screening service.*

Trevigen Cell Assays
Custom Contract Services

## Quotation Request Form - Comet Assay

First and Last Name _____

Email Address _____

Company _____

Street Address _____

City _____ State/Province _____

Zip/Postal Code _____ Country _____

Telephone Number _____ Fax Number _____

Which type of Comet Assay is required? (Alkaline or Neutral) _____

How many samples do you have to screen? _____

How many replicates are required? _____

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

A) What types of cells are required? _____

B) Will you be providing the cells? _____

If you are preparing the slides and only require analysis, how many slides will be analyzed? 2-well _____ 20-Well _____

What is the desired reporting format? _____

How soon is the data required? _____

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

_____

**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 diluent 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.

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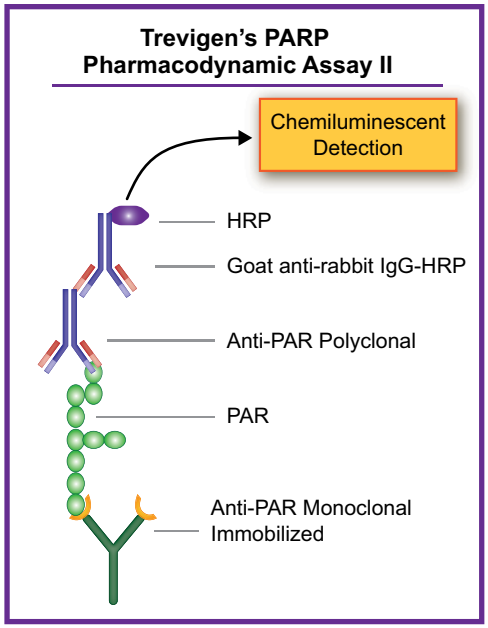
**FORM TCA 2 DATE** _____


*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.*

# HT PARP in vivo Pharmacodynamic Assay

## The PDA II kit is for RESEARCH USE ONLY

PARP catalyzes the NAD+-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* Pharmaco-dynamic Assay II employs a 96 well plate, pre-coated with Trevigen's monoclonal PAR antibody as the capture agent, and anti-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-HR with chemiluminescent detection.
· Light output (Signal) correlates with the amount of cellular PAR.

| | Catalog No. | Application(s) | Detection Method | Test Sample | Sensitivity |
|---|---|---|---|---|---|
| **PARP in vivo Pharmacodynamic Assay II** · Chemiluminescence Detection | PARP Inhibition 4520-096-K | PAR Ab in vivo PAR Levels in Lysates | In vivo PARylation | PMBC Lysates Tissue Lysates | 2-1000 pg/ml PAR |

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**FORM TCA 2 DATE** _____

# HT PARP in vivo Pharmacodynamic Assay

*Getting started is easy. Fax the completed form to 301-560-4973; or email us at TCA@trevigen.com; or complete the form online at www.trevigencellassays.com with the information that we need in order to set up your screening service.*

Trevigen Cell Assays
Custom Contract Services

## Quotation Request Form - PDAII 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 by 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 solubility of 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.

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**FORM TCA 3 DATE** _____


*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+ is consumed for the synthesis of highly negatively charged polymers of ADP-ribose (PAR) on target nuclear proteins that include PARP 1 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+ 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 $IC_{50}$ 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. PARP1, 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 |
|---|---|---|---|---|---|
| **Universal PARP Assay Kits** | | | Biotin NAD | PARP | 0.01-1 Units |
| • Colorimetric Detection | 4677-096-K | PARP Inhibitor Screen | Histone | Inhibitors | PARP |
| • Chemiluminescent Detection | 4676-096-K | $IC_{50}$ Determination | ribosylation | | |
| **Homogeneous PARP Inhibition Assay** | | HT PARP | NAD Consumption | PARP | 10% PARP |
| • Fluorescence Detection | 4690-096-K | Inhibitor Screen | PARP ribosylation | Inhibitors | Inhibition |
| **PARP/Apoptosis Assay Kits** | | PARP Inhibitor Screen | PAR Ab | PARP | 0.1-10 mUnits |
| • Colorimetric Detection | 4684-096-K | PARP Activity | Histone | Inhibitors | PARP |
| • Chemiluminescent Detection | 4685-096-K | in Lysates | ribosylation | Cell Lysates | 500 cells |
| **PARG Assay Kits** | | | | | |
| • Colorimetric Detection | 4683-096-K | PARG Inhibitor | Biotin NAD | PARG | 250 pg |
| • Chemiluminescent Detection | 4682-096-K | Screen | PAR degradation | Inhibitors | PARG |
| **Homogeneous PARG Inhibition Assay** | | PARG Inhibitor | | | 10% PARG |
| • Chemiluminescent Detection | 4691-096-K | Screen in vitro | | | Inhibition |
| **PARP in vivo Pharmacodynamic Assay II** | | See FORM TCA 2 for PDAII questionnaire | | | |

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**FORM TCA 3 DATE** _____

# PARP/PARG Assay

*Getting started is easy. Fax the completed form to 301-560-4973; or email us at TCA@trevigen.com; or complete the form online at www.trevigencellassays.com with the information that we need in order to set up your screening service.*

Trevigen Cell Assays
Custom Contract Services

## 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. #** _____

*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 solubility of 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.

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**FORM TCA 4 DATE** _____


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# Cultrex® Cell Invasion and Migration Assays

## Cultrex® Cell Invasion Assays

Cultrex® Cell Invasion Assays were created in an effort to accelerate the screening process for compounds that influence cellular degradation of and migration across extracellular matrices, which is a fundamental component of cellular processes such as angiogenesis, embryonic development, immune responses, and tumor cell metastasis. 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.

These assays employ a simplified Boyden chamber design with a polyethylene terephthalate (PET) membrane containing 8 micron pores, which allow access from the input chamber (top) to the assay chamber (bottom) without dismantling the device. The assay chamber may be directly analyzed in a 96-well plate reader, eliminating transfer steps that introduce additional variability to the assay.

Cell invasion is quantified using calcein-acetoxymethyl ester (Calcein-AM), which is internalized by the cells, and cleaved by intracellular esterases to generate fluorescence signals. In conjunction with a standard curve, these signals may be used to quantitate the number of cells that have migrated or invaded – thereby eliminating the need for direct cell counting. Since different cell lines and different treatments can result in a wide range of invasive potentials, the permissiveness of each matrix may also be optimized to fit each experiment by adjusting the coating concentration. Cultrex® Cell Invasion Assays provide multiple formats for evaluation against different extracellular matrices and matrix components: Laminin I, Collagen I, Collagen IV, and Basement Membrane Extract (BME).

For your convenience and for greater reproducibility, Trevigen introduced invasion kits with pre-coated wells.

## Cultrex® Cell Migration Assays

The Cultrex® Cell Migration Assays utilize uncoated Boyden chambers to assess cell migration in the absence of extracellular proteins.

| Assay | Coating | Catalog # |
|---|---|---|
| Invasion | BME | 3455 |
| Invasion | Laminin I | 3456 |
| Invasion | Collagen I | 3457 |
| Invasion | Collagen IV | 3458 |
| Migration | None | 3465 |

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**FORM TCA 4 DATE** _____

# Cultrex® Cell Invasion and Migration Assays

Trevigen Cell Assays
Custom Contract Services

*Getting started is easy. Fax the completed form to 301-560-4973; or email us at TCA@trevigen.com; or complete the form online at 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 - Cell Invasion/Migration 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 is required? ☐ Cell Invasion – Cat. # _____ ☐ Migration – Cat. # _____

*Please check the appropriate assay and write in the catalog #. If unsure of the proper kit to use, please contact us and we can aid you in identifying the proper kit for your needs.*

How many samples do you have to screen? _____

How many replicates are required? _____

How many compounds and concentrations do you have to screen? _____

What type of cells are required? _____

Will you be providing the cells? _____

What is the desired reporting format? _____

How soon is the data required? _____

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

**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 diluent is required? _____

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**FORM TCA 5 DATE**

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# DNA Repair Gene Knockdown Cell Lines

## Tools to study genomic instability and genotoxic stress

DNA repair proteins maintain the stability of the genome. When repair protein function is impaired through mutations, the genome may become unstable which is a hallmark of solid tumors. The availability of this panel of knockdown cell lines will permit scientists to study the molecular etiology of tumor genomic instability and to exploit it in oncology research. The Knockdown line encompasses the Base Excision Repair pathway, Non-Homologous End Joining, Mismatch Repair and Homologous Recombination pathways.

**Base Excision Repair** - PARP1, OGG1, NTHL1, NEIL2, NEIL3, UNG, SMUG1, MPG, MutYH, Neil1, PARP2, PARP3, XRCC1, APE1, APE2, TDG, MBD4, LIG3

**Mismatch Repair** - MSH2, PMS2, MLH3, MSH3, MLH1, MSH5, MSH6

**Non-homologous End Joining** - SIRT5, SIRT1, PRKDC, SIRT2, XRCC6 (Ku70), XRCC5 (Ku80), SIRT3

**Homologous Recombination** - RAD54B, NBS1, RAD21, XRCC3, SMC6L1, SHFM1, BRCA1, RAD51C, RAD50

Trevigen's Knockdown Cell Lines are target specific LN428 (glioblastoma) shRNA lentivirus transduced cells. They are rigorously qualified and mycoplasma free. The percent knockdown levels range from 63-98% depending on the gene, as evaluated by RT-PCR. Lentiviruses are maintained by puromycin selection.

## Features

- Tested negative for Mycoplasma
- Easy to use Glioblastoma based knock down cells with reliable short and long term knock down efficiency.
- Tested negative for: Human immunodeficiency virus ( HIV1, HIV2), Hepatitis viruses (A, B, and C), Human T-lymphotropic virus (HTLV 1, HTLV 2), Epstein Barr, Hantaviruses Hantaan (Seoul, Sin, Nombre), Herpes simplex (1+2) Human cytomegalovirus, Hurman herpes virus (6+8), Human adenovirus, Varicella virus and Lymphocytic Choriomeningitis virus.
- Control Cell line is available.

When purchased, cell lines are provided under a material transfer agreement. When done as a service project, the material transfer agreement is not required.

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**FORM TCA 5 DATE** _____

# DNA Repair Gene Knockdown Cell Lines

*Getting started is easy. Fax the completed form to 301-560-4973; or email us at TCA@trevigen.com; or complete the form online at www.trevigencellassays.com with the information that we need in order to set up your screening service.*

## Quotation Request Form - Knockdown Cell Lines

First and Last Name _____

Email Address _____

Company _____

Street Address _____

City _____ State/Province _____

Zip/Postal Code _____ Country _____

Telephone Number _____ Fax Number _____

What KD cell lines? _____

Which Assays? *(if the assay is already offered please complete the appropriate form)* _____

- Other *(specify)* _____

**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? _____

_____

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.

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