TREVIGEN® Instructions

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DNA Repair Gene Knockdown Cell Lines

Cat# 55XX-001-01
Cat# 55XXX-001-01

Tools to Study Genomic Instability and Genotoxic Stress

interchim

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* **Limited Use Notice**: Trevigen's DNA Repair Gene Knockdown Cell Lines can only be obtained under a Materials Transfer Agreement executed with Trevigen, Inc. The Materials Transfer Agreement conveys to the buyer the non-transferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer, in their laboratory only. For details on the Materials Transfer Agreement or for information on purchasing a license for these products for purposes other than research, contact Trevigen, Inc.

I. Introduction

DNA repair pathways usually maintain the integrity of the genome [1], thereby reducing the onset of cancer, disease and aging phenotypes. Conversely, the requirement for DNA repair and genome maintenance in response to radiation and genotoxic chemotherapeutics, implicates DNA repair proteins as prime targets for improving responses to currently employed anti-cancer regimens [2]. Further, cancer-specific DNA repair defects offer novel approaches for tumor selective therapy [3]. There are over 150 human proteins with functional roles in DNA repair. These fall into 14 general categories, including the classical DNA Repair pathways of Base Excision Repair (BER) [4], Direct Reversal of Damage, Mismatch Excision Repair (MMR), Nucleotide Excision Repair (NER), Homologous Recombination (HR), Non-Homologous End-Joining (NHEJ) and the Fanconi Anemia/DNA crosslink repair pathway, plus proteins that modulate nucleotide pools, DNA polymerases, editing and processing nucleases.

Table 1: Base Excision Repair (BER) Knockdowns

Target	KD Cell Line	Cat. Number	%KD by RT-PCR
N/A	KD-BER-LN428-Control	5503-001-01	N/A
APE1	KD-BER-LN428-APE1	5517-001-01	90%
APE2	KD-BER-LN428-APE2	5518-001-01	80%
BRACA-1	KD-HR-LN428-BRCA1**	5502-001-01	83%
MBD4	KD-BER-LN428-MBD4	5506-001-01	72%
MPG	KD-BER-LN428-MPG	5511-001-01	98%
MutYH	KD-BER-LN428-MutYH	5512-001-01	87%
NEIL1	KD-BER-LN428-NEIL1	5513-001-01	92%
NEIL2	KD-BER-LN428-NEIL2	5507-001-01	86%
NEIL3	KD-BER-LN428-NEIL3	5508-001-01	95%
NTHL1	KD-BER-LN428-NTHL1	5505-001-01	91%
OGG1	KD-BER-LN428-OGG1	5504-001-01	63%
PARG	KD-BER-LN428-PARG	5501-001-01	84%
PARP1	KD-BER-LN428-PARP1	5500-001-01	72%
PARP2	KD-BER-LN428-PARP2	5514-001-01	83%
PARP3	KD-BER-LN428-PARP3	5515-001-01	70%
SMUG1	KD-BER-LN428-SMUG1	5510-001-01	63%
TDG	KD-BER-LN428-TDG	5519-001-01	74%
UNG	KD-BER-LN428-UNG	5509-001-01	87%
XRCC1	KD-BER-LN428-XRCC1	5516-001-01	81%

**BRCA1 participates in the Homologous Recombination Pathway and demonstrates synthetic lethality in combination with PARP1.

In order to study the DNA repair response to genotoxic stress Trevigen is now offering a collection of knockdown (KD) cell lines each deficient in a DNA repair gene mRNA transcript (e.g. Table 1). Cell lines harboring a unique shRNA lentivirus targeted to a specific DNA repair gene mRNA transcript were constructed using the LN428 glioma cell line. The percent knockdown is reported as the percent reduction of the targeted transcript in reference to a control cell line.

II. Precautions and Limitations

1. Successful and consistent results are dependent upon the quality and degree of characterization of the cells under investigation. Highly passaged cells may undergo both genotypic and phenotypic changes that render them an inadequate *in vitro* model for specific investigations. We recommend for all studies that highly qualified low passage number cells are used to ensure reliable and reproducible results.
2. For Research Use Only. Not for use in diagnostic procedures.
3. This cell line is not known to harbor any agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least a Biosafety Level 1 containment. This cell line has NOT been screened for Hepatitis B, human immuno-deficiency viruses or other adventitious agents. Trevigen recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Trevigen assumes no liability for damage resulting from handling or contact with these products.

III. Materials Supplied

For a complete list of currently available DNA repair gene knockdown lines, please see: http://www.trevigen.com/cat/1/1/0/DNA_Repair_Gene_Knockdown_Cell_Lines/

Component	Quantity	Storage	Catalog #
BER KD Cell Lines	1 Vial (10^6 Cells)	Liquid Nitrogen***	55XX-001-01
Non BER KD Lines	1 Vial (10^6 Cells)	Liquid Nitrogen***	55XXX-001-01

***Shipped on Dry Ice, immediately thaw for use, or for long term storage place in vapor phase of liquid nitrogen.

IV. Materials/Equipment Required But Not Supplied

Equipment

1. 1 - 20 µl, 20 - 200 µl, and 200 - 1000 µl pipettors
2. Laminar flow hood or clean room
3. 37°C CO_2 incubator
4. 37°C Water Bath
5. Hemocytometer or other means to count cells
6. Inverted standard or phase microscope
7. Pipette aid
8. Liquid Nitrogen Storage
9. Low speed swinging bucket centrifuge and tubes for cell harvesting
10. Cell freezing container that allows for slow freezing of cells (e.g. Fisher Scientific cat#15-350-50)

Reagents

1. Cell Culture Medium: α-MEM (Mediatech cat# 10-022CV or equivalent)
2. Cell Harvesting Reagent, Trypsin, Dispase, etc.
3. Heat Inactivated (HI) Fetal Bovine Serum
4. L-Glutamine (200 mM)
5. 100X Antibiotic/Antimycotic supplement for Media (Life Technologies cat# 15240-062 or equivalent)
6. Gentamycin (10 mg/ml solution)
7. Puromycin
8. PBS (Mg^{2+}, Ca^{2+} free) or HBSS, tissue culture grade
9. Trypan blue or equivalent viability stain
10. DMSO, tissue culture grade
11. 70% Ethanol
12. Sterile ddH_20

Disposables

1. Cell culture flasks, 25 cm^2, 75 cm^2, or 185 cm^2
2. 15 ml tubes
3. 0.22 µm Filter Unit (optional)
4. 1 - 200 µl and 200 - 1000 µl pipette tips
5. 2, 5 and 10 ml serological pipettes
6. gloves

V. Reagent Preparation

These procedures should be performed in a biological hood utilizing aseptic technique to prevent contamination.

1. Knockdown (KD) Basal Growth Medium

For 500 ml of Medium:

α-MEM Medium:	438.5 ml
Heat Inactivated Fetal Bovine Serum	50.0 ml
200 mM L-Glutamine:	5.0 ml
10 mg/ml Gentamycin	2.5 ml
100X Antibiotic/Antimycotic Solution	4.0 ml

Filter sterilize medium and store at 4°C for one month. Ensure medium is at room temperature or 37°C prior to use. Note: Due to presence of the antibiotic gentamycin the final concentration of 100X Antibiotic/ Antimycotic is less than 1X.

2. Puromycin

Dissolve Puromycin powder to 50 mg/ml in sterile ddH_20.
Aliquot 10 µl into sterile tubes and freeze at -20°C.

Thaw 1 aliquot and add 90 µl of sterile ddH_20 to the tube for a 5 mg/ml Puromycin solution (store any unused material at 4°C for up to 1 week).

Add 10 µl of 5 mg/ml Puromycin solution to 50 ml of Knockdown Basal Growth Medium (final concentration is 1 µg/ml) just before use to make Knockdown Complete Growth Medium.

3. 2X Freeze Medium

For 10 ml:

KD Basal Growth Medium:	4.0 ml
HI FBS:	4.0 ml
DMSO:	2.0 ml

VI: Protocol

These procedures should be performed in a biological hood utilizing aseptic technique to prevent contamination. Vessels should be sprayed down with 70% ETOH before placing in Tissue Culture Hood.

A. Thawing KD Cells:

1. Prepare Complete Growth Medium on day of use. Prewarm Knockdown Basal Growth Medium (Section V.1) to 37°C by placing in H_2O bath or in Tissue Culture Incubator. Aliquot 15 ml of Basal Growth Medium into a 50 ml conical tube and add Puromycin (V.2) (final concentration of 1 µg/ml).
2. Immediately before use, remove the vial of cryopreserved KD cells from liquid nitrogen freezer and thaw quickly in a 37°C H_2O bath. Ensure cells are completely thawed before proceeding and do not leave cells at 37°C past thawing.
3. Aseptically, transfer the thawed cells to an empty 15 ml conical tube. Wash ampoule with 1 ml of warm Complete Growth Medium and add to thawed cells. Add 1 ml of warm Complete Growth Medium to 15 ml conical tube containing cells, gently swirling to mix between drops. Total Volume should be 3 ml.
4. Centrifuge cells at 200 x g for 3 minutes.
5. Remove supernatant gently to avoid disturbing cell pellet and resuspend cell pellet in 10-12 ml of fresh Complete Growth Medium.
6. Transfer cell suspension to a sterile T75 Tissue Culture Flask.
7. Place Tissue Culture Flask/Dish in 5% CO_2 Tissue Culture Incubator at 37°C.
8. Change medium in flasks using freshly prepared and prewarmed Complete Growth Medium the next day.

B. Passaging KD Cells:

1. Medium should be changed every 2-3 days. Cells should be passaged when 90-100% confluent for optimal growth rate/efficiency. We recommend splitting cells at a density of 1:6 to 1:8 from a confluent flask. Cells can be split out as far as a 1:10 dilution. Cells have a doubling time of approximately 38 hrs.
2. Prepare Complete Growth Medium on day of use. Warm Basal Growth Medium to 37°C by placing in 37°C H_2O bath or in Tissue Culture Incubator. In a sterile container add the required volume (10-12 ml/flask) of Basal Growth Medium and Puromycin (final concentration of 1 µg/ml).

3. If replacing medium, remove spent medium from T75 flask containing the KD cells and replace with 10-12 ml of fresh Complete Growth Medium.

4. If splitting cells, remove medium from T75 flask containing the KD Cells.
   a. Gently wash flask with 5-10 ml of sterile 1X PBS (Ca^{2+} and Mg^{2+} free) and remove PBS.
   b. Add 2 ml of prewarmed Trypsin to each flask and place at 37°C for 3-5 minutes until cells are no longer attached to plate. Add 4 ml of Basal Growth Medium to flask to inactivate Trypsin and transfer to 15 ml conical tube.
   c. Centrifuge cells at 200 x g for 3 minutes.
   d. Remove supernatant gently to avoid disturbing cell pellet and resuspend cell pellet in 6 ml of fresh Complete Growth Medium (if splitting 1:6) or 8 ml of Complete Growth Medium (if splitting 1:8).
   e. Add 1 ml of cell suspension to 9-11 ml of Complete Growth Medium and transfer to T75 flask.

C. Freezing Cells

1. In general, one confluent T75 flask flask will provide cells for 10-12 vials of 5 x 10^5 cells per vial.

2. Prepare 2X Freeze Medium, (see Section V.3) according to volume required. Typically, 0.5 ml of 2X Freeze Medium is mixed with 0.5 ml of 1x10^6 cells.

3. To prepare cells remove medium from T-75 flask containing KD Cells.
   a. Gently wash flask with 5-10 ml of sterile 1X PBS (Ca^{2+} and Mg^{2+} free) and remove PBS.
   b. Add 2 ml of prewarmed Trypsin to each flask, and place at 37°C for 2-3 minutes until cells are no longer attached to the plate. Add 4 ml of Basal Growth Medium to flask to inactivate Trypsin and transfer to 15 ml conical tube.
   c. Centrifuge cells at 200 x g for 3 minutes.
   d. Remove supernatant gently to avoid disturbing cell pellet and resuspend cell pellet in 2 ml of Basal Growth Medium.

4. Count cells on hemocytometer (per standard protocol) and dilute cells to 1 x 10^6 cells/ml in Basal Growth Medium.

5. Add equal volume of 2X Freeze Medium to the cells, mix gently and aliquot 1 ml of cells into labeled cryovials.

6. For initial storage, first place cryovials on ice for 15-30 minutes. Transfer cells to specialized cell freezing container and place in -80°C freezer overnight.

7. For long term storage, transfer cells (next day) to liquid nitrogen freezer to ensure long-term viability.

VII. References

1. Friedberg EC, Walker GC, Siede W, Wood RD, Schultz RA, Ellenberger T. 2006. DNA Repair and Mutagenesis, 2nd Edition. Washington, D.C: ASM Press.

2. Ljungman M 2009. Targeting the DNA damage response in cancer. *Chem Rev* **109**:2929-2950.

3. Peralta-Leal A, Rodriguez MI, Oliver FJ. 2008. Poly(ADP-ribose)polymerase-1 (PARP-1) in carcinogenesis: potential role of PARP inhibitors in cancer treatment. *Clin Transl Oncol,* **10**:318-323

4. Almeida KH, Sobol RW. 2007. A unified view of base excision repair: lesion-dependent protein complexes regulated by post-translational modification. DNA Repair, **6**:695-711

VIII. Troubleshooting

PROBLEM	CAUSE	ACTION
Poor viability from initial freeze	Improper thawing of cells	Ensure medium is added slowly to re-equilibrate the KD cells from freeze medium Ensure cells were removed from freeze medium immediately after vial has been thawed Ensure vial of cells was thawed at 37°C Fresh medium was prewarmed to 37°C
Poor proliferation	Fetal Bovine Serum not optimal for KD cell growth	Try alternative lot/source of HI FBS
	Media not optimal for KD cell growth	Ensure medium is of the proper formulation
	Frequency of medium change	Ensure medium is changed every 2-3 days Ensure pH of medium fresh medium has not changed
	CO_2 incubator not humidified	Add sterile water to CO_2 incubator per manufactures instructions
	No gas exchange is allowed by flask	Ensure cap is loosened to allow air gas or use vented flask
Contamination of Cells	Contaminated Medium	To prevent contamination, filter medium through a 0.22 µm filter before use *Never use contaminated medium once cloudy or after microorganisms are visible under the microscope*
	Improper aseptic technique	Spray down hands, reagents and hood with 70% ethanol before opening any flasks
	Hood is working improperly	Check to make sure blower is on and functioning Ensure hood is currently certified Wipe down hood with 70% ethanol
	Contaminated CO_2 Incubator	Ensure CO_2 incubator is free of microbial growth
Loss of KD "expression"	Not growing cells in Puromycin	Ensure Puromycin was added to basal medium just before addition to cells
	Frequency of Medium Change	Ensure medium is being changed every 2-3 days.

IX. Related Products Available From Trevigen

Contact Trevigen for details of our unique product line for studying DNA damage and repair. All of Trevigen's kits include highly qualified enzymes, substrates, buffers, full instructions for use, and a synopsis specific for your kit.

DNA Repair Enzymes:

Catalog #	Description	Size
4055-100-EB	T4 Endonuclease V (T4-Pyrimidine Dimer Glycosylase/ T4-PDG)	100,000 U
4150-010-EB	*Sulfolobus silfataricus* DNA Polymerase IV (Dpo4)	10 µg
4045-O1K-EB	*E. coli* Endonuclease III	1000 U
4050-100-EB	*E. coli* Endonuclease IV	100 U
4130-100-EB	Human 8-oxo-G DNA Glycosylase (hOGG1)	100 U
4040-100-EB	*E. coli* Fpg	500 U
4025-100-EB	*E. coli* Uracil-N-Glycosylase	100 U
4000-500-EB	*E. coli* MutY DNA Glycosylase	500 U
4110-01K-EB	Human AP Endonuclease	1000 U
4020-01K-EB	Human β Polymerase	1000 U
4120-100-EB	Human FEN-1	100 U
4125-100-EB	*E. coli* Mismatch Uracil DNA Glycosylase (Mug)	100 U
4090-100-EB	Mouse 3-Methyladenine DNA Glycosylase (Aag)	100 U
4070-500-EB	Thermostable thymine mismatch DNA Glycosylase	500 U
4100-100-EB	*S. pombe* Ultraviolet DNA Endonuclease (UVDE)	100 µl
4065-100-EB	Chorella Virus Pyrimidine Dimer Glycosylase	1000 U

CometAssay® Kits:

Catalog #	Description	Size
4250-050-ES	CometAssay® Electrophoresis System (ES)	each
4250-050-ESK	CometAssay® ES Starter Kit	each
4250-050-K	CometAssay® Kit	50 samples
4252-040-K	CometAssay® HT	40 samples
4251-050-K	CometAssay® Silver Kit	50 samples
4254-200-K	CometAssay® Silver Staining Kit	200 samples
4252-040-K	CometAssay® Higher Throughput Kit	40 samples
4253-096-K	CometAssay® Kit 96 Wells	96 samples

DNA Damage Antibodies:

Catalog #	Description	Size
4411-PC-100	γ-H2AX polyclonal	100 µl
4410-PC-100	Fen-1 polyclonal	100 µl
4350-MC-100	UVssDNA mAb (clone C3B6)	100 µg
4354-MC-50	Anti-8-oxo-dG mAb (clone 2E2)	50 µl
4335-MC-100	Anti-PAR polymer mAb (clone 10HA)	100 µl
4336-BPC-100	Anti- PAR polymer polyclonal	100 µl
4338-MC-50	Anti-human/murine-PARP mAb (clone C2-10)	50 µg

FLARE™ Assay Kits:

Catalog #	Description	Substrate	Size
4040-100-FK	Fpg Kit	8-oxoguanine	75 samples
4055-100-FK	T4-PDG Kit	Cis-syn isomers of cyclobutane pyrimidine dimers	75 samples
4065-100-FK	cv-PDG Kit	Cis-syn and trans-syn isomers of cyclobutane pyrimidine dimers	75 samples
4130-100-FK	hOGG1 Kit	8-oxoguanine, DNA containing formamidopyrimidine moieties	75 samples
4100-100-FK	UVDE Kit	Cyclobutane pyrimidine dimers, (6-4) photoproducts	75 samples
4045-01K-FK	Endonuclease III Kit	Thymine Glycol, 5,6-dihydrothymine urea, 5-hydroxy-6-hydrothymine, 5,6-dihydrouracil, alloxan, 5-hydroxy-6-hydrouracil, uracil glycol, 5-hydroxy-5-methylhydantoin, 5-hydroxycytosine,5-hydroxyuracil, methyltartonyl urea, thymine ring saturated or fragmentation product	75 samples

Oxidative Damage Quantitation:

Catalog #	Description	Size
4380-096-K	HT 8-oxo-dG ELISA kit II	96 tests

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