TREVIGEN®

Trevigen, Inc. is a rapidly growing biotechnology company focused on the development of products and technology for cancer research, emphasizing apoptosis, DNA damage and repair, and cancer cell function and behavior. Trevigen has been a long-standing provider of quality reagents and kits for researchers investigating programmed cell death and DNA damage and repair. A logical extension of our focus on cancer research has been the recent development of assays for cancer cell function and behavior including angiogenesis, cell invasion and tumor formation. Currently, our product portfolio contains over 500 products categorized into four processes – *Apoptosis, DNA Damage and Repair, Angiogenesis, and Oxidative Stress.*

www.trevigen.com **1.800.873.8443**

interchim

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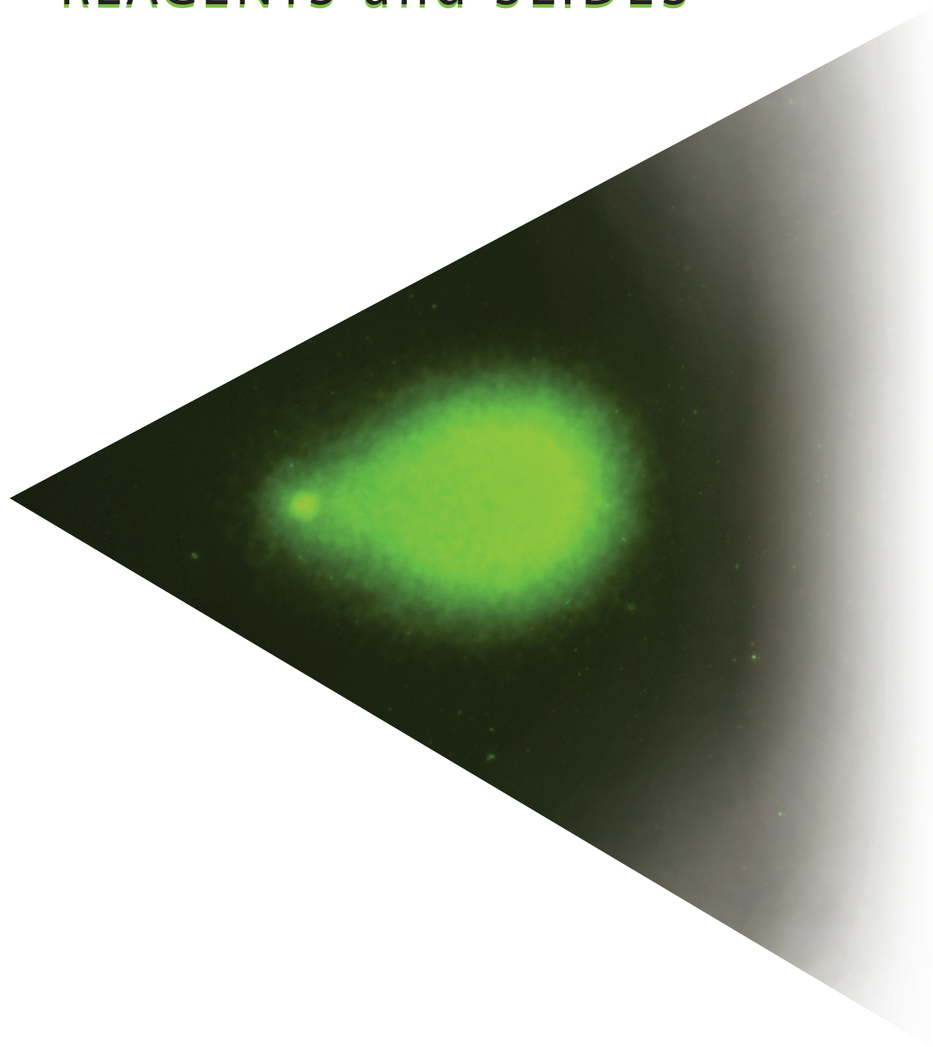
CometAssay™ KITS
REAGENTS and SLIDES



TREVIGEN®

CometAssay™ PRODUCTS

CometAssay™

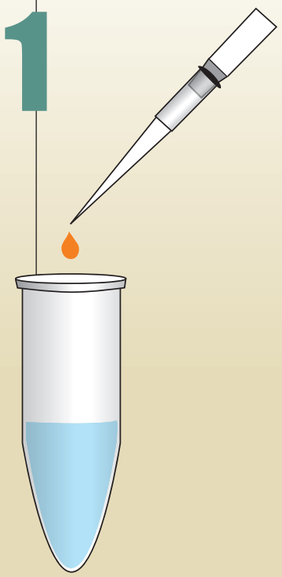
For the direct measurement of DNA Damage

The ability of chemical substances, isolated from samples of outdoor and indoor airborne particulates, to induce mutagenicity in prokaryotic cells and eukaryotic cells is well studied. These chemicals have been found to interact with the vital tissue macromolecules regulating the cellular functions leading to long lasting health disorders. Acute and chronic exposure to several of these environmental chemicals such as pesticide, metals, polycyclic aromatic hydrocarbons (PAHs), solvents etc. have been shown to produce marked toxicity at the target sites. Some of these chemicals affect the DNA, which is the carrier of inherited information and any gross change in its structure potentiates serious biological changes. Hence there is a need to test the chemicals for their genotoxic potential before being released into the environment. The conventional methods for evaluating genetic damage include chromosomal aberration, micronucleus assay and sister chromatid exchanges. However these are time consuming, resource intensive and require proliferating cell population. Hence newer and more sensitive test systems have now been introduced for assessing the genotoxicity of chemicals.

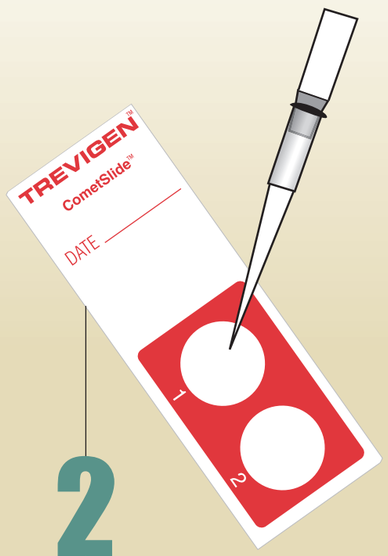
The single cell gel electrophoresis or CometAssay™ is one such state-of-the-art technique for quantitating DNA damage and repair from *in vivo* and *in vitro* samples of eukaryotic cells and some prokaryotic cells. This technique is rapid, non-invasive, sensitive, visual and inexpensive compared to conventional techniques and is a powerful tool to study factors modifying mutagenicity and carcinogenicity. It is the only technique that directly measures DNA damage in individual cells and as a result has rapidly gained importance in the fields of genetic toxicology and human biomonitoring. 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.

1

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



2



Immobilize cells on CometSlide™

3

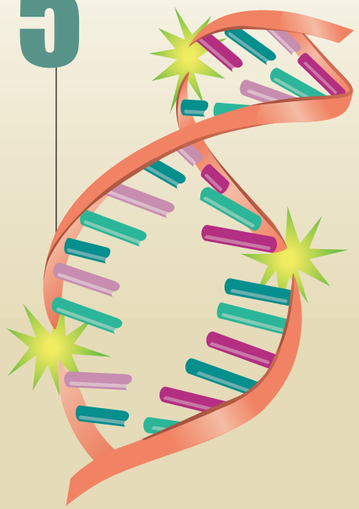
Treat cells with Lysis Solution (removes membranes and histones from the DNA)

4

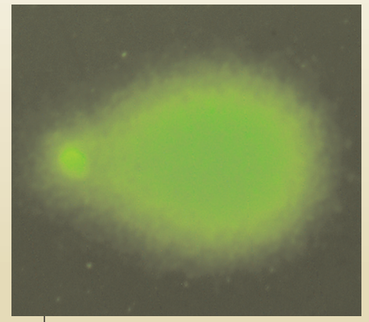
Samples treated with alkali (unwinds and denatures DNA)

5

Alkaline electrophoresis performed (reveals DNA breaks)

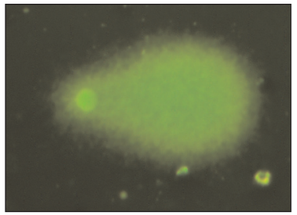


6



Samples stained with intercalating dye and visualized by epifluorescence microscopy

CometAssay™ Kits



Trevigen's CometAssay™ provides reagents and our exclusive CometSlide™ for the rapid analysis of DNA fragmentation associated with DNA damage. Following alkaline lysis, the unwound, relaxed DNA is able to migrate out of the cell during electrophoresis and can be visualized using SYBR® Green I nucleic acid gel stain. Cells that have accumulated DNA damage appear as fluorescent comets with tails of DNA fragmentation or unwinding, whereas, normal undamaged DNA does not migrate far from the origin. The CometAssay™ is provided with our exclusive CometSlides™ which greatly simplify the assay. Each slide provides surfaces specially treated to promote agarose adherence, and a hydrophobic barrier to allow treatment with one of Trevigen's DNA repair enzymes. Simply add your cells to the low melting point Comet LMAgarose, and pipet onto the slide.

2 Well CometSlide™

20 Well CometSlide™

CometAssay™ Kits

Description	Size	Catalog No.
CometAssay™ Kit	50 samples	4250-050-K
CometAssay™ Silver Kit	50 samples	4251-050-K
CometAssay™ HT Sample Kit	40 samples	4252-040-K
CometAssay™ 96 Well Kit	96 samples	4253-096-K

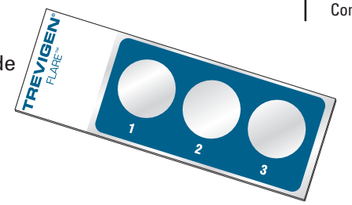
CometAssay™ Reagents

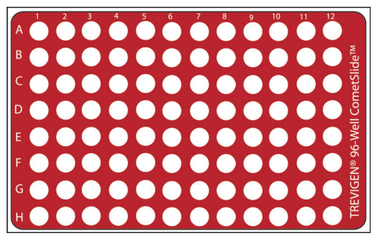
Description	Size	Catalog No.
CometAssay™ Lysis Solution	2 x 500 ml	4250-050-01
CometAssay™ LMAgarose	15 ml	4250-050-02
200 mM EDTA; pH 10	12.5 ml	4250-050-04
SYBR® Green	5 µl	4250-050-05

CometAssay™ and FLARE™ Slides

Description	Size	Catalog No.
CometSlide™ (2 well slide)	25 slides	4250-050-03
CometSlide™ (2 well slide)	100 slides	4250-200-03
CometAssay™ HT Slide (20 well slide)	25 slides	4252-500-01
CometAssay™ HT Slide (20 well slide)	100 slides	4252-02K-01
96 Well CometSlide™ (96 well side)	10 slides	4253-960-03
96 Well CometSlide™ (96 well side)	20 slides	4253-02K-03
96 Well CometSlide™ (96 well side)	100 slides	4253-10K-03
FLARE™ Slides (3 well slide)	25 slides	3950-075-02
FLARE™ Slides (3 well slide)	100 slides	3950-300-02
CometSlide™ Rack System	each	4252-040-02

FLARE™ Slide





96 Well CometSlide™

FLARE™ Assays and Slides

Trevigen's unique FLARE™ (Fragment Length Analysis using Repair Enzymes) Assays provide the ability to detect DNA damage in single cells using a variety of DNA repair enzymes in conjunction with Trevigen's CometAssay™ single gel electrophoresis kit. To assess the type of DNA Damage induced by putative mutagen, drug, or treatment regimen, cells are harvested after treatment and immobilized in a layer of low melting point agarose on the FLARE™ slide. Trevigen's three well FLARE™ Slide can be interchanged with the CometSlide™. FLARE™ slides allow direct application of LMAgarose without base layers or pretreatment for 3 samples. See related products on page 14 for further details.

PRODUCTS

CometAssay™ Control Cells: For the standardization of Comet assays

Trevigen's CometAssay™ Control Cells are a set of cell preparations containing different levels of DNA damage to be used with Trevigen's CometAssay™ Kits. In a typical comet assay, electrophoresis methods and differences in cell preparations create a significant source of variation in comet tail parameters. Such variation sometimes makes it difficult to compare results between laboratories, and even within the same lab. To overcome this problem, Trevigen scientists developed a set of stable control cell populations containing incremental levels of DNA damage for use when performing the CometAssay™. These control cells, when electrophoresed in the CometAssay™, consistently produce four distinct populations. The healthy control cell population (CC0) was treated with Etoposide under various conditions to increase the amount of damage in the three different populations - CC1, -CC2, and -CC3, respectively. These cryopreserved control cells are designed to act as controls to standardize and compare alkaline electrophoresis methods between individual users and laboratories.

CometAssay™ Control Cells

Description	Size	Catalog No.
CometAssay™ Control Cells	1 Set (10 Assays)	4256-010-CC

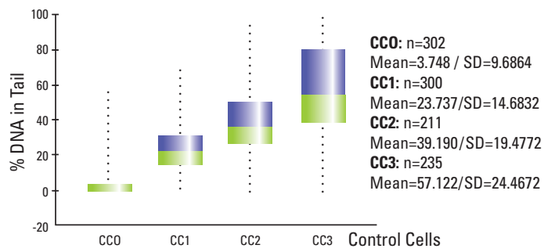
CometAssay™ Control Cells Contents

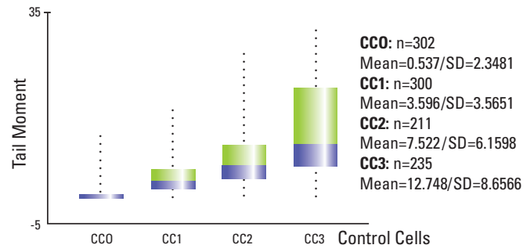
Component	Quantity	Catalog No.
Healthy Cells	500 µl	4256-010-CC0
Treated Cells - Level 1	500 µl	4256-010-CC1
Treated Cells - Level 2	500 µl	4256-010-CC2
Treated Cells - Level 3	500 µl	4256-010-CC3

Features & Benefits

Features	*Benefits*
• First available set of qualified control cells for use with the CometAssay™	• Standardize and validate alkaline electrophoresis methods between individual users and laboratories
• Includes four populations: one population of healthy cells and three populations with increasing amounts of DNA damage	• Provides a range of DNA damage for comparison, which meets the needs of most laboratories. Damaged populations show statistically distinct differences
• Cells are cryopreserved	• After initial thaw, aliquot and refreeze, the cells remain stable in liquid nitrogen for long term storage
• Qualified for alkaline gel electrophoresis	• Compatible with the electrophoresis method used by most laboratories

Damage Measurement for Control Cells





Quality Control Specifications - To ensure that our control cells allow for standardization of your CometAssay™ results within your lab and between different labs, see our ranges listed on page 5 in RESULT ANALYSIS.

RESULT ANALYSIS

Definitions

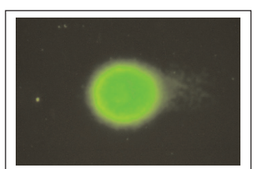
Percent DNA in the Tail - The integrated tail intensity x 100 divided by the total integrated cell intensity for a normalized measure of the percent of total cell DNA found in the tail.

Tail Moment - The product of distance and normalized intensity integrated over the tail length, Σ (Lx • % DNAx); a damage measure combining the amount of DNA in the tail with the distance of migration (severity of damage).

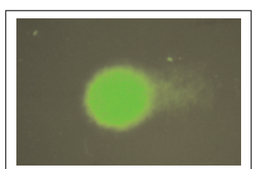
CometAssay™ Control Cells

Component #	Description	% DNA in Tail*
4256-010-CC0	Healthy Cells	0-10%
4256-010-CC1	Treated Cells – level 1	11-30%
4256-010-CC2	Treated Cells – level 2	31-50%
4256-010-CC3	Treated Cells – level 3	>50%

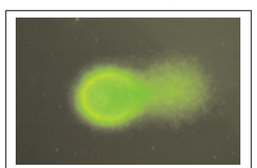
*Measurements obtained using Trevigen's CometAssay™ protocol with Loats Associates, Inc. Automated Comet Assay Analysis System.



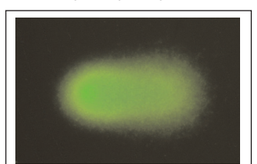
CCO - Healthy Cells
0-10% DNA in Tail



CC1 - Treated Cells
11-30% DNA in Tail



CC2 - Treated Cells
31-50% DNA in Tail



CC3 - Treated Cells
>50% DNA in Tail

WHAT CUSTOMERS SAY

Larry Gladnick
Pharmaceutical Scientist

"I initially purchased the CometAssay™ Control Cells for the purpose of training others to manually score Comet cells. The slides were straightforward to prepare, and were an excellent addition to the training regime used for my colleagues. Not only are the 4 different types of control cells significantly different when comparing % tail DNA, they are also easily differentiated under the scope or with image analysis. This was an added benefit for training. In the future I will use the CometAssay™ Control cells as additional controls in our in vitro/in vivo Comet studies to ensure study reproducibility between trials and individual experimenters."

Avinash M. Tope, PhD
Principal Investigator
Kentucky State University

"We perform CometAssay™ routinely at the Human Health and Nutrition Research, Kentucky State University, and are interested in investigating DNA protective capacity of bioactive compounds found in vegetables and fruits. We have been using various Trevigen products including the Fpg FLARE™ kit. We are pleased with their performance quality and services."

Randa El-Zein, MD, PhD
The University of Texas
M.D. Anderson
Cancer Center

"I have been using the Trevigen CometAssay™ reagents and slides for several years and have found the products to be excellent in terms of quality, reproducibility and pricing. In addition the products come with an impressive customer support service and excellent technical competence. Trevigen has been quick to respond to our technical needs and have been critical to our success in developing new markers for risk assessment."

CITATIONS

Recent Citations using Trevigen CometAssay™ Products –
Product descriptions and catalog numbers on pages 3 and 4

The CC Chemokine Ligand 2 (CCL2) Mediates Fibroblast Survival through IL-6

Xiangde Liu, Anuk M. Das, Jonathan Seideman, Don Griswold, Chantal N. Afuh, Tetsu Kobayashi, Shinji Abe, Qiuhong Fang, Mitsu Hashimoto, Huijung Kim, Xingqi Wang, Lei Shen, Shin Kawasaki, and Stephen I. Rennard
Am. J. Respir. Cell Mol. Biol., Jul 2007; 37: 121 – 128
...were considered apoptotic cells. **CometAssay™** was performed using a commercially available kit (**Trevigen**, Gaithersburg, MD). Briefly, HFL-1...harvested by trypsinization and used for comet assay following the manufacturers instruction...

Ethonafide-Induced Cytotoxicity Is Mediated by Topoisomerase II Inhibition in Prostate Cancer Cells

Alan Pourpak, Terry H. Landowski, and Robert T. Dorr
J. Pharmacol. Exp. Ther., Jun 2007; 321: 1109 – 1117.
...determined by **neutral comet single-cell gel electrophoresis** (**CometAssay™ kit**; **Trevigen**, Gaithersburg, MD) following...**cold lysis solution** (**Trevigen**) containing 80 mug/ml...dilution of **SYBR® Green I** (**Trevigen**). DNA was visualized...captured per sample, and the comet moment was calculated...

Regulation of Bcl-xL Expression in Lung Vascular Smooth Muscle

Yuichiro J. Suzuki, Hiroko Nagase, Chi Ming Wong, Shilpashree Vinod Kumar, Vivek Jain, Ah-Mee Park, and Regina M. Day
Am. J. Respir. Cell Mol. Biol., Jun 2007; 36: 678 – 687.
...The neutral comet assay was used to measure double-stranded DNA...in PBS, embedded in 1% agarose, and placed on a **CometSlide™** (**Trevigen**). Cells were then placed in a lysis solution...

Suicide Gene Therapy of Human Colon Carcinoma Xenografts Using an Armed Oncolytic Adenovirus Expressing Carboxypeptidase G2

Silke Schepelmann, Lesley M. Ogilvie, Douglas Hedley, Frank Friedlos, Janet Martin, Ian Scanlon, Ping Chen, Richard Marais, and Caroline J. Springer **Cancer Res.**, May 2007; 67: 4949 – 4955.
...comet assay. Assays were done in...analyzed using the **CometAssay™ kit** (**Trevigen**) and CometScore...infection), and analyzed using the comet assay. Controls consisted of...single cell gel electrophoresis comet assay. In untreated cells...

Resveratrol increases vascular oxidative stress resistance

Zoltan Ungvari, Zsuzsanna Orosz, Aracelie Rivera, Nazar Labinskyy, Zhao Xiangmin, Susan Olson, Andrej Podlutsky, and Anna Csiszar **Am J Physiol Heart Circ Physiol**, May 2007; 292: H2417 - H2424.
...DNA damage analysis by comet assay. Endothelial cells were...spotted on **CometAssay™ slides** (**Trevigen**, Gaithersburg, MD) between two...single-cell electrophoresis (comet assay), according to the modified...Tice et al. (47). Briefly, the comet assay is based on the alkaline...

CITATIONS

Recent Citations using Trevigen CometAssay™ Products –
Product descriptions and catalog numbers on pages 3 and 4

Fanconi anemia pathway–deficient tumor cells are hypersensitive to inhibition of ataxia telangiectasia mutated

Richard D. Kennedy, Clark C. Chen, Patricia Stuckert, Elyse M. Archila, Michelle A. De la Vega, Lisa A. Moreau, Akiko Shimamura, and Alan D. D'Andrea **J. Clin. Invest.**, May 2007; 117: 1440 – 1449.
…comet assay. EUFA326 or EUFA326G cells…was measured using the neutral **Trevigen CometAssay™ kit** as per the manufacturers…instructions. Percentage DNA in comet tails was scored for 100 comets…The mean percentage DNA per comet tail SEM was calculated using…

Inflammatory ROS promote and cooperate with the Fanconi anemia mutation for hematopoietic senescence

Xiaoling Zhang, Daniel P. Sejas, Yuhui Qiu, David A. Williams, and Qishen Pang
J. Cell Sci., May 2007; 120: 1572 – 1583.
…single-cell gel electrophoresis (comet) assay (Fairbairn et al., 1995…analysis using repair enzymes) **CometAssay™ kit** in accordance with the manufacturer's instructions (**Trevigen**, Gaithersburg, MD). For each…100 cells were evaluated for comet-tail length from each culture…

Integrated molecular profiling of SOD2 expression in multiple myeloma

Elaine M. Hurt, Suneetha B. Thomas, Benjamin Peng, and William L. Farrar
Blood, May 2007; 109: 3953 – 3962.
…counting. 2ME2 treatment and Comet assay Cells (106) were treated…assessed using the **CometAssay™ kit** (**Trevigen**, Gaithersburg, MD). Document…description of the procedure. Comet assays were visualized and photographed…includes DNA damage, we performed Comet assays on cell lines with differing…

Effect of pressure at primary drying of freeze-drying mouse sperm reproduction ability and preservation potential

Yosuke Kawase, Toshio Hani, Nobuo Kamada, Kou-ichi Jishage, and Hiroshi Suzuki
Reproduction, Apr 2007; 133: 841 – 846.
…the shape of the DNA comet tail and migration pattern…suspension was suspended in **Comet LMAgarose** (1% low-temperature melting agarose, **Trevigen**, Gaithersburg, MD, USA…stained with **SYBR® Green** (**Trevigen**), and analyzed under…spermatozoa was assessed by comet assay twice per experimental…

BRCA1 Contributes to Cell Cycle Arrest and Chemoresistance in Response to the Anticancer Agent Irofulven

Timothy Wiltshire, Jamie Senft, Yutian Wang, Gregory W. Konat, Sharon L. Wenger, Eddie Reed, and Weixin Wang
Mol. Pharmacol., Apr 2007; 71: 1051 – 1060.
… The **CometAssay™** (**Trevigen Inc.**, Gaithersburg, MD) was performed according…75 mul (500-1000 cells) was immediate ly added to **CometSlide™**. After hardening, slides were incubated for…

JS-K, a GST-activated nitric oxide generator, induces DNA double strand breaks, activates DNA damage response pathways, and induces apoptosis *in vitro* and *in vivo* in human multiple myeloma cells

Tanyel Kiziltepe, Teru Hideshima, Kenji Ishitsuka, Enrique M Ocio, Noopur Raje, Laurence Catley, Chun-Qi Li, Laura J Trudel, Hiroshi Yasui, Sonia Vallet, Jeffery L Kutok, Dharminder Chauhan, Constantine S. Mitsiades, Joseph E Saavedra, Gerald N Wogan, Larry K Keefer, Paul J Shami, and Kenneth C. Anderson **Blood**, Mar 2007; 10.1182/blood-2006-10-052845.
…Neutral single-cell gel electrophoresis (**CometAssay™**). Neutral comet assays were performed using the **Trevigen CometAssay™ kit** to asses JS-K-induced DNA double-strand breaks. Cells at 1 x 10^5 /mL were combined…

Effects of the Chemotherapy Cocktail Used to Treat Testicular Cancer on Sperm Chromatin Integrity

Géraldine Delbes, Barbara F. Hales, and Bernard Robaire
J Androl, Mar 2007; 28: 241 – 249.
…for every sample. **CometAssay™** DNA strand breaks…evaluated using the alkaline comet assay, as previously…evenly spread onto slides (**Trevigen**, Gaithersburg, MD…**SYBR® Green solution** (**Trevigen**) (1:10 000 in Tris-EDTA…images were scored for comet parameters. Tail length…

High Levels of Heat Shock Protein Hsp72 in Cancer Cells Suppress Default Senescence Pathways

Julia A. Yaglom, Vladimir L. Gabai, and Michael Y. Sherman
Cancer Res., Mar 2007; 67: 2373 – 2381.
…Alkaline **CometAssay™** was done according…manufacturers protocol (**Trevigen**, Helgerman, CT…analyzed by alkaline **CometAssay™** according to the manufacturers protocol (**Trevigen**). B, depletion of Hsp72…

8-Oxoguanine DNA Glycosylase and MutY Homolog Are Involved in the Incision of Arsenite-Induced DNA Adducts

Yeong-Shiau Pu, Kun-Yan Jan, Tsing-Cheng Wang, Alexander S. S. Wang, and Jia-Ran Gurr
Toxicol. Sci., Feb 2007; 95: 376 – 382.
…breaks, as analyzed by a standard Comet assay. However, breaks were detected…glycosylase (Fpg) were purchased from **Trevigen** (Gaithersburg, MD). T4 UV endonuclease…and (CH3)2AsOH (DMAIII). **CometAssay™**. The standard Comet assay without enzyme digestion…

Human RAD18 is involved in S phase-specific single-strand break repair without PCNA monoubiquitination

Naoko Shiomi, Masahiko Mori, Hideo Tsuji, Takashi Imai, Hirokazu Inoue, Satoshi Tateishi, Masaru Yamaizumi, and Tadahiro Shiomi **Nucleic Acids Res.**, Jan 2007; 35: e9.
…5-GCGGCCATTGTCCGTCAGGACATTGTTGGA-3. **CometAssay™** (single cell micro gel electrophoresis) The alkaline comet assay was performed using a **CometAssay™ kit** (**CometAssay™; Trevigen**) according to the manufacturer's protocol…

Essential Roles for Fe65, Alzheimer Amyloid Precursor-binding Protein, in the Cellular Response to DNA Damage

Giuseppina Minopoli, Maria Stante, Francesco Napolitano, Francesca Telese, Luigi Aloia, Mario De Felice, Roberto Di Lauro, Roberto Pacelli, Arturo Brunetti, Nicola Zambrano, and Tommaso Russo
J. Biol. Chem., Jan 2007; 282: 831 – 835.
…12 h before changing medium. **CometAssay™** -The neutral comet assay was carried out according to manufacturer's recommendations (**Trevigen**, Gaithersburg, MD). Slides were…alkaline solution (pH 13), and comet tails were generated by a 10 min…

Products referenced in Citations

Catalog No.	Product	Description
4040-100-EB	*E. coli* Fpg Enzyme and Buffer	Fpg releases damaged bases preferentially from duplex DNA. It has an associated class I AP lyase activity, leaving both 3' and 5' phosphoryl groups. This results from a β, δ elimination reaction at the AP sites, producing a single nucleotide gap in the DNA. The enzyme consists of 269 amino acids with a molecular weight of 30.2 kDa.
4040-100-FK	*E. coli* Fpg. FLARE™ Kit	Trevigen's Fpg FLARE™ (Fragment Length Analysis using Repair Enzymes) Assays provide the ability to probe for oxidative damage in conjunction with Trevigen's CometAssay™ single cell gel electrophoresis kit. To assess the type of DNA damage induced by a putative mutagen, drug, or treatment regimen, cells are harvested after treatment and immobilized in a layer of low melting point agarose on the FLARE™ Slide.
4045-01K-EB	*E. coli* Endonuclease III Enzyme and Buffer	Endonuclease III releases damaged bases induced by UV, ionizing radiation, osmium tetroxide, or acid. It is a DNA glycosylase with an associated AP lyase activity and contains aniron-sulfur group which helps to maintain its three dimensional conformation. Endonuclease III cleaves as a DNA lyase at abasic sites by β-elimination, producing a single nucleotide gap in the DNA. The enzyme has a molecular weight of 23.4 kDa and is suitable for use in FLARE™.
4250-050-K	CometAssay™ Kit	Trevigen's CometAssay™ provides reagents and our exclusive CometSlide™ for the rapid analysis of DNA fragmentation associated with DNA damage. The unwound, relaxed DNA is able to migrate out of the cell during electrophoresis and can be visualized using SYBR® Green I nucleic acid gel stain. Cells that have accumulated DNA damage appear as fluorescent comets with tails of DNA fragmentation or unwinding, whereas, normal undamaged DNA does not migrate far from the origin.
4250-050-01	CometAssay™ Lysis	CometAssay™ Lysis Solution is provided with the CometAssay™ Kit to gently lyse the cells.
4250-050-02	CometAssay™ LMAgarose	CometAssay™ LMAgarose is provided with the CometAssay™ Kit. The cells are mixed with the LMAgarose and pipeted onto the CometSlides™.
4250-200-03	CometSlides™	CometSlides™ greatly simplify the comet assay by providing a sample surface specifically treated to promote agarose adherence and a hydrophobic barrier to allow treatment with one of Trevigen's DNA repair enzymes.
4250-050-05	CometAssay™ SYBR®	SYBR® Green I nucleic acid gel stain is provided in the CometAssay™ Kit. Cells that have accumulated DNA damage appear as fluorescent comets with tails of DNA framentation or unwinding, whereas, normal undamaged DNA does not migrate far from the origin.
4350-MC-100	UVssDNA Antibody	DNA exposed to UV light accumulates a number of DNA photoproducts, predominantly (6-4) photoproducts and cyclobutane pyrimidine dimers. Isolated DNA, intact cells or tissues may be investigated using fluorescent immunohostochemical methods. The antibody is ideal for studies involving UV damage to skin or UV damage DNA repair mechanism and is suitable for FLARE™.
6300-100-K	DePsipher™ Mitochondrial Potential Assay	The mitochondrial permeability transition is an important event in the apoptotic process wherein the electrochemical gradient (referred to as Dym) across the mitochondrial membrane collapses. The DePsipher™ Kit uses a unique cationic dye (5,5'6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) to indicate the loss of the mitochondrial potential. The dye readily enters cells and fluoresces bright red in its multimeric form within healthy mitrochondria. In apoptotic cells, the mitochondrial membrane potential collapses, and the DePsipher™ reagent cannot accumulate within the mitochondria. In these cells, DePsipher remains in the cytoplasm as a green fluorescent monomeric form. Apoptotic cells, showing primarily green fluorescence, are easily differentiated from healthy cells which show red fluorescence.

FAQS

1. Will the CometAssay™ (4250-050-K) work with solid tissue? If so how?

a) Carefully prepared cell suspensions from tissues can be used with the CometAssay™.

Protocol Guideline

i. Place a small piece of tissue in 1-2 ml of cold HBSS containing 20 mM EDTA.

ii. Mince tissue into fine pieces and allow settling.

iii. Remove 5-10 µl of the supernatant (cell suspension) and combine with 75 µl molten LMAgarose.

Note: To ensure the cell concentration and dissociation are sufficient, a small aliquot can be diluted in PBS (instead of LMAgarose) and spotted onto a microscope slide. Another option is to digest the tissue for 15 minutes with trypsin, wash with HBSS or cold PBS containing serum to inactivate the enzyme and then mechanically dissociate using syringe and needle.

2. Are there recommendations to reduce SYBR® (4250-050-05) Green I fading?

a) Apply diluted SYBR® Green I solution and stain for 30 minutes in the dark. Decant stain and dry to completion in the dark before viewing to avoid quenching (fading).

b) Apply additional SYBR® Green I solution without washing off first application.

c) Avoid prolonged exposure to fluorescent light from microscope.

d) Prepare Anti-fade Solution, as described, and apply 10 µl per sample, covering samples with coverslip. In a 50 ml tube, mix until dissolved: 500 mg p-Phenylenediamine dihydrochloride 4.5 ml 1X PBS. Add approximately 400 µl of 10 N NaOH dropwise with stirring until pH of solution reaches 7.5-8.0. Add 1X PBS to increase the volume to 5 ml, and 45 ml of glycerol for a final volume of 50 ml. Vortex mixture thoroughly and apply 10 µl per sample, covering samples with coverslip. Nail polish may be used to seal coverslip. Re-staining of slides is not recommended. Store anti-fade solution at -20°C for up to one month. Darkening of solution may occur.

e) Add beta-mercaptoethanol (1 mM) to diluted dye.

3. Is it possible to combine the Anti-fade and SYBR® (4250-050-05) Green I Solutions?

a) The solutions can only be mixed prior to application. Precipitation occurs upon storage of Anti-fade and SYBR® Green I mixture.

4. Do you recommend use of the CometAssay™ (4250-050-K) with whole blood?

a) We do not recommend using whole blood with the CometAssay™ because sample preparation is critcial and hemoglobin could damage DNA. It's important to note that mammalian red blood cells (major blood component) do not have a nucleus (i.e. genomic DNA) and therefore not suitable for use with CometAssay™.

FAQS

5. How long can the CometSlide™ (4250-050-03) be stored before applying SYBR® Green I Staining Solution?

a) Slides immersed in 70% ethanol for 5 min and dried can be stored at room temperature with dessicant for 1 year prior to staining.

6. What is the optimal filter set for SYBR® Green I (4250-050-05)?

a) SYBR® Green I's (4250-050-05) maximum excitation and emission are 494 and 521 nm, respectively. Fluorescein filter is adequate.

7. What image analysis software is available for scoring comets?

a) **PC software:** Popular software is available from the CometAssay™ software project at: http://casp.sourceforge.net Free software also available from: http://autocomet.com This software automatically calculates seventeen different parameters.

b) **Mac software:** NIH Image, free software for image analysis, is available at http://rsb.info.nih.gov/nih-image. This software can be used along with calculation macros specifically designed to give comet tail moment. A useful macro for calculating the tail moment from digital images has been provided by Professor Herbert M. Geller, Ph.D, and is available through http://www2.umdnj.edu/~geller/lab/comet.htm.

c) Listing of image analysis packages is available on CometAssay™ Interest Group website at: http://cometassay.com/image_analysis_software.htm

8. Why is there no staining in the positive control?

a) Verify retention of the agarose sample on the slide. Initial application of agarose sample should cover the entire well on your slide. Once dry there is a 0.5 mm clear ring separating the agarose from the edge of the well, as the agarose will shrink back.

b) Verify that ~1000 cells were present in agarose sample applied to the well.

c) Use fresh aliquot of hydrogen peroxide to create positive control.

9. What is the importance of pH for the electrophoresis buffer?

a) The answer will depend upon adducts under analysis. At pH 12.1, initial breaks are analyzed, while at pH 12.5 and pH 13 alkaline labile adducts are converted to breaks. At pH 12.5, abasic lesions are converted to single strand breaks and at pH 13, additional labile sites are converted to single and double strand breaks. Maximum damage caused by an agent is visualized at pH 13 in CometAssay™ and FLARE™ Assay.

10. Why are negative controls showing more DNA damage than expected in adherent cells?

a) For trypsin incubations, incubate in 2% cold Trypsin for 30 minutes. Centrifuge for 10 minutes at speeds less than 200xg to avoid damage. Cold EDTA (2 mM) can be used instead of Trypsin.

FAQS

11. What is the effect of light on the CometAssay™ (4250-050-K)?

a) The samples can be handled under normal laboratory lights but the Lysis and Alkali Unwinding Steps should be performed in the dark. Electrophoresis is typically performed under normal laboratory lights also. Use of dim yellow light for very sensitive applications has also been reported. (Chan, K.F., Siu, S.Y.M., Mclella, K.E., Tse, A.K.W., Lau, B.M.F., Nikezic, D., Richardson, B.J., Lam, P.K.S. Fong, W.F., and Yu, K.N. 2006. Alpha-particle radiobiological experiments using thin CR-39 Detectors. Radiation Protection. 10.1093/rpd/ncl393.)

12. Will a ZEISS Axioplan/Axiovert microscope work for viewing a CometSlide™ (4250-200-03)? Will any commercial horizontal gel electrophoresis device work for electrophoresis?

a) The ZEISS Axioplan/Axiovert microscope will work for viewing a CometSlide™. Yes, most commercial electrophoresis chambers can be used but they are adjusted using cold electrophoresis buffer to setup a current between the electrodes at 1 Volt per cm. (If there are 30 cm between electrodes, set the current at 30V). Electrophoresis is performed at constant voltage. The buffer level in the chamber is just above the agar and electrophoresis is performed for 20-30 minutes.

13. How long can a CometSlide™ (4250-200-03) be stored?

a) Prior to staining, dried slides stored with desiccant can be kept for extended periods (months). Using the CometAssay™ Silver Staining Kit, (4251-050-K) permanent records are created and visualization using standard light microscopy is possible.

14. Does it matter if the same pH is used for alkali unwinding and alkaline electrophoresis?

a) The same solution (> pH 13) is used for alkali unwinding and alkaline electrophoresis. To avoid variation due to pH, the buffers should be prepared fresh. At pH 12.1, initial breaks are analyzed, while at pH 12.5 and pH 13 alkaline labile adducts are converted to breaks. At pH 12.5, abasic lesions are converted to single strand breaks and at pH 13, additional labile sites are converted to single and double strand breaks. Maximum damage caused by an agent is visualized at pH 13 in CometAssay™ and FLARE™ Assay.

15. Is it possible to differentiate between apoptotic and necrotic cells using the CometAssay™?

a) Listed below are two references discussing differentiation of apoptotic and necrotic cells using a derivation of the CometAssay™. Please also see the Trevigen DASH™ Kit Cat# 4225-050-K.

i) Fairbairn, D. W., Walburger D. K, Fairbairn J. J and O'Neill K. L. Key morphologic changes and DNA strand breaks in human lymphoid cells: discriminating apoptosis from necrosis. Scanning 1996 Sep: 18(6):407-16

ii) Singh, N. P., A simple method for accurate estimation of apoptotic cells. Exp Cell Res 2000 Apr 10; 256(1):328-37

FAQS

16. Why are comet tails in positive control cells smaller than expected?

a) It may be necessary to extend the electrophoresis time period. Recommended time period is 20 to 40 minutes.

b) Verify lysis of cells. Lysis Solution should be chilled prior to use. Lysis Solution will precipitate upon long term storage at 4°C.

17. Is it necessary to use a coverslip with a CometSlide™ (4250-200-03)?

a) The Trevigen CometSlide™ was designed to be used without coverslips because removal of the coverslip could lift agarose and cause damage to the samples. In order to reduce fading, coverslips are used after the application of Anti-fade Solution. Restaining of slides is not recommended after application of Anti-fade Solution and coverslip.

18. The high throughput comet slide (CometSlide™ HT) has 20 sample wells; is it possible to re-use the slide if not all wells were utilized?

a) This is not recommended since the slides contain a special coating for binding the agarose, which can be compromised if washed for re-use.

19. Are there protocols for removing the plant cell wall prior to performing the CometAssay™ (4250-050-K)?

a) The CometAssay™ group website (http://cometassay.com) provides references and protocols for performing the CometAssay™ with plant cells.

b) Two additional references (untried by Trevigen) are: 1) Wang, C., and Liu, Z. 2006. Arabidopsis Ribonucleotide Reductase Are Critical for Cell Cycle Progression, DNA Damage Repaire, and Plant Development. The Plant Cell. 18: 350-365. and 2) Li, N., Zhang, D., Liu, H., Yin, C., Li, X., Liang, W., Yuan, Z., Xu, B., Chu, H., Wang, J., Wen, T., Huang, H., Luo, D., Ma, H., Zang, D. 2006. The Rice Tapetum Degradation and Anther Development. The Plant Cell. 18: 2999-3014.)

20. The protocol for the CometAssay™ Silver Staining Kit (4251-050-K) is designed for two well slides. Can the 100 µl volumes be decreased for twenty well slides?

a) Yes, the volumes can be reduced to 50 µl.

21. Do you have information or reference using CometAssay™ (4250-050-K) for measuring oxidant stress in blood other than red blood cells?

a) Sardas S, Yilmaz M, Oztok U, Cakir N, Karakaya AE. Assessment of DNA strand breakage by CometAssay™ in diabetic patients and the role of antioxidant supplementation. Mutat Res. 2001 Feb 20; 490(2): 123-9.

b) Peter Moller and Steffen Loft, Oxidative DNA damage in human white blood cells in dietary antioxidant intervention studies. American Journal of Clinical Nutrition, Vol. 76, No. 2, 303-310, August 2002.

RELATED PRODUCTS

Researchers who purchased CometAssay™ products also purchased these...

Product	Size	Catalog No.
8-oxo-dG Oligo and Complement A	100 pmol	3850-100-OL
8-oxo-dG Oligo and Complement B	100 pmol	3851-100-OL
FLARE™ Slides (3 well slide)	25 slides	3950-075-02
FLARE™ Slides (3 well slide)	100 slides	3950-300-02
Human DNA Polymerase ß Enzyme & Buffer	500 units	4020-100-EB
Human DNA Polymerase ß Kit	100 units	4020-100-EB
E.coli Fpg Enzyme & Buffer	500 units	4040-100-EB
E.coli Fpg FLARE™ Kit	75 samples	4040-100-FK
E.coli Endonuclease III Enzyme & Buffer	1000 units	4045-01K-EB
E.coli Endonuclease III FLARE™ Kit	75 samples	4045-01K-FK
E.coli Endonuclease IV Enzyme & Buffer	100 units	4050-100-EB
T4 Endonuclease Enzyme & Buffer	100,000 units	4055-100-EB
T4 Endonuclease FLARE™ Kit	75 samples	4055-100-FK
cv-PDG Enzyme & Buffer	1,000 units	4065-100-EB

Product	Size	Catalog No.
cv-PDG FLARE™ Kit	75 samples	4065-100-FK
S. pombe UVDE Enzyme & Buffer	100 µl	4100-100-EB
S. pombe UVDE FLARE™ Kit	75 samples	4100-100-FK
Mismatched Uracil Gylcosylase Enzyme & Buffer	100 units	4125-100-EB
hoGG1 Enzyme & Buffer	100 Units	4130-100-EB
hoGG1 FLARE™ Kit	75 samples	4130-100-K
Anti-PAR Polymer Monoclonal Antibody	100 µl	4335-MC-100
AntiPAR Polymer Polyclonal Antibody	100 µl	4336-BPC-100
Anti-8-oxo-dG Antibody	50µg	4354-MC-050
HT Universal Color PARP Assay Kit w/ Histone Reagents	96 samples	4671-096-K
HT Universal Chemiluminescent PARP Assay Kit w/ Histone Reagent	96 Samples	4675-096-K
HT Universal Color PARP Assay Kit w/ Histone Coated Strip Wells	96 Samples	4677-096-K
Streptavidin-HRP	30 µl	4800-30-06