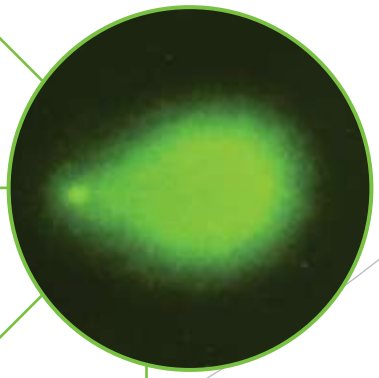
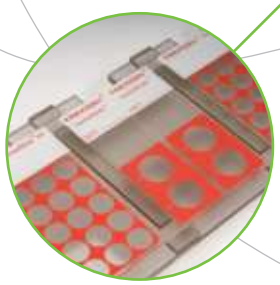
STANDARDIZED CometAssay™ SYSTEM

Instrumentation

Control Cells





Slides

Kits

amsbio

info@amsbio.com

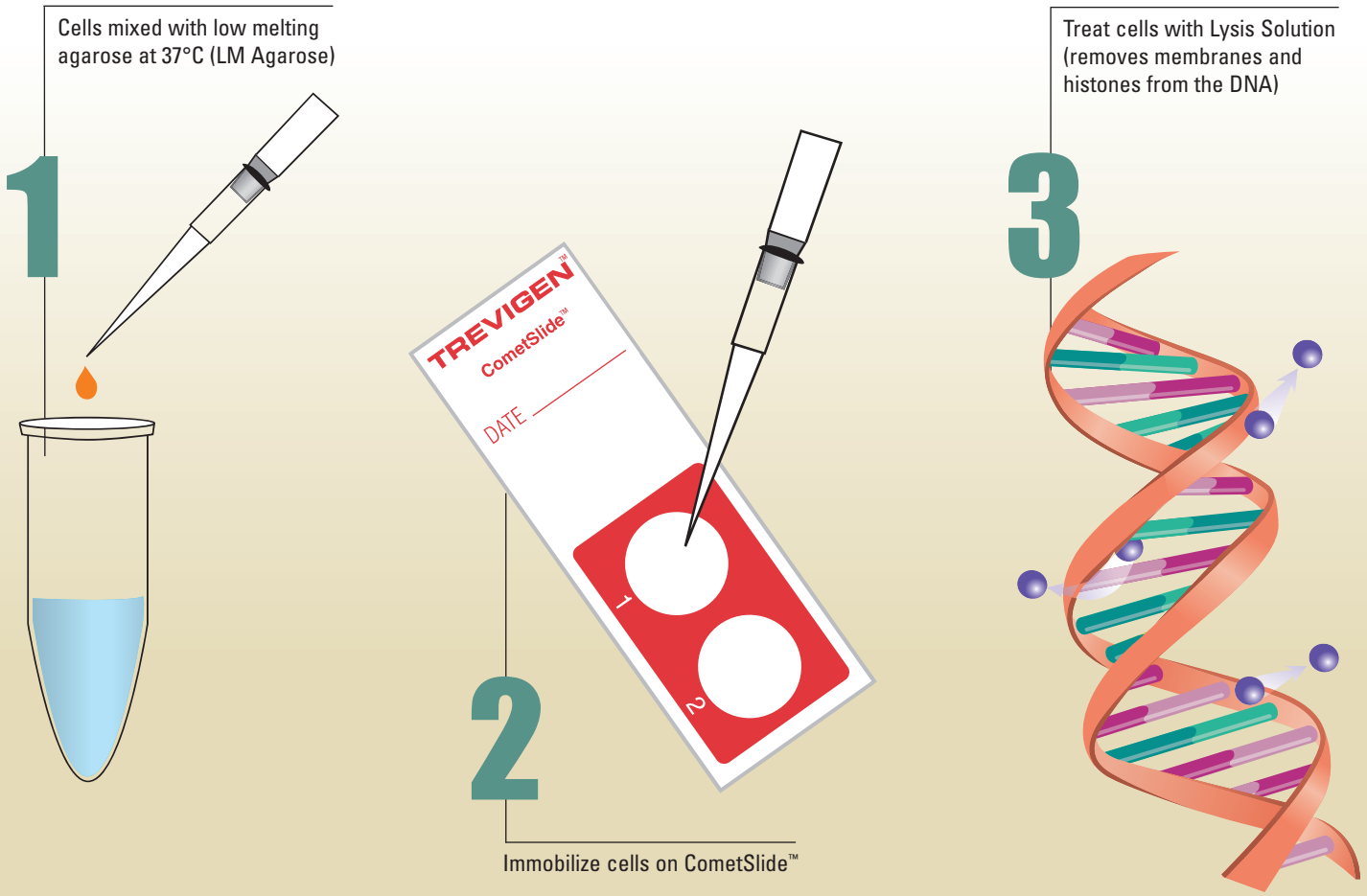
TREVIGEN®

CometAssay™ PRODUCTS

CometAssay™

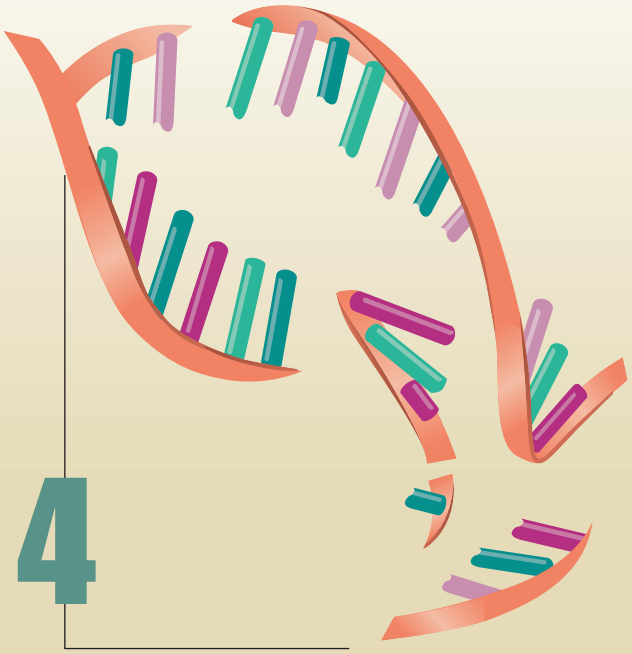
For the direct measurement of DNA Damage

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.



4

Samples treated with alkali
(unwinds and denatures DNA)

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

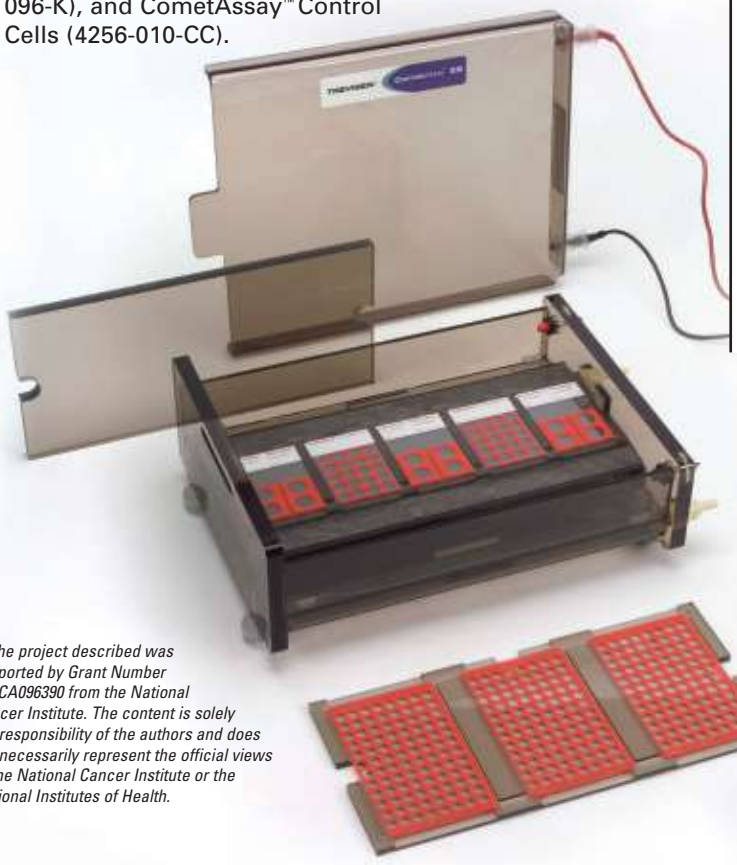
5

NEW COMPLETE STANDARDIZED SYSTEM

CometAssay™ Electrophoresis System

The standardized CometAssay™ Electrophoresis System enables investigators to consistently optimize alkaline comet assays for highly reproducible results, and to standardize alkaline electrophoresis methods between individual users and laboratories. The comet assay is the only direct method for the detection of DNA damage in cells. It is used in cancer research, in genotoxicity studies on environmental mutagens, and for screening compounds for cancer therapeutics. The comet assay can be variable depending on temperature, distance between electrodes, and buffer height. Trevigen, with funding from Phase II SBIR grants**, has solved these problems and makes available a novel complete assay system which includes CometAssay™ kits, CometSlides™, Comet Assay™ control cells and a specialized electrophoresis unit. This unit retains test cells in a uniquely configured electrophoretic field permitting consistent DNA migration patterns, which are critical for standardization of the assay. Each lot of the CometAssay™ control cells, reagents and CometSlides™ developed by Trevigen are tested and qualified for use in the CometAssay™ System.

Products are available in a System Starter Kit (4250-050-ESK) or as individual components listed in the table to the right. CometAssay™ Electrophoresis System Starter Kit includes the Electrophoresis System (4250-050-ES), a CometAssay™ Kit, with choice of 2-well, 20-well, or 96-well CometSlides™(4250-050-K, 4252-040-K, 4253-096-K), and CometAssay™ Control Cells (4256-010-CC).



***The project described was supported by Grant Number R44CA096390 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.*

CometAssay™ System

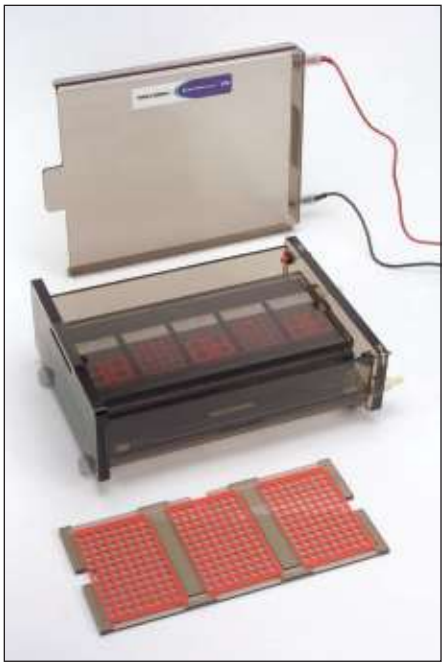
Instrumentation		Catalog No.
CometAssay™ Electrophoresis System Starter Kit		4250-050-ESK
CometAssay™ Electrophoresis System Starter Kit + PS*		4250-050-ESK-PS1
CometAssay™ Electrophoresis Unit		4250-050-ES
Kits	**Size**	**Catalog No.**
CometAssay™ Kit	50 samples	4250-050-K
CometAssay™ Silver Kit	50 samples	4251-050-K
CometAssay™ HT Sample Kit	40 samples	4252-040-K
CometAssay™ 96 Well Kit	96 samples	4253-096-K
Reagents	**Size**	**Catalog No.**
CometAssay™ Lysis Solution	2 x 500 ml	4250-050-01
CometAssay™ LM Agarose	15 ml	4250-050-02
200 mM EDTA; pH 10	12.5 ml	4250-050-04
SYBR® Green	5 µl	4250-050-05
CometSlides	**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 slide)	10 slides	4253-960-03
96 Well CometSlide™ (96 well slide)	20 slides	4253-02K-03
96 Well CometSlide™ (96 well slide)	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
Control Cells and Contents	**Size**	**Catalog No.**
CometAssay™ Control Cells	1 Set (10 Assays)	4256-010-CC
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

**PS = Power Supply*

INSTRUMENTATION

CometAssay™ Electrophoresis Unit

Trevigen's CometAssay™ ES overcomes variations by placing an acrylic overlay on top of an elevated slide tray to maintain optimal buffer height for DNA migration. A constant buffer temperature is maintained using an underlying water jacket to cool the ceramic slide platform and buffer chamber. Notice the outlet valves for the water jacket on the right side of the image. Specially designed slide trays are provided to accommodate 2, 20, and 96 well slides and maintain proper slide orientation in an electrophoretic field uniquely configured for single cell gel electrophoresis.



Features

- Maintains constant buffer temperature.
- Maintains optimal buffer level for consistent results.
- Specially designed trays accommodate 2, 20 and 96 well slides and maintain correct position during electrophoresis.
- Optimized for use with CometAssay Kits and CometAssay Control Cells.
- Greater reproducibility and reduced variablity between individual users and different labs

CometAssay™ Electrophoresis Unit

Description	Catalog No.
CometAssay™ Electrophoresis Unit	4250-050-ES

KITS

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 LM Agarose, and pipet onto the slide.

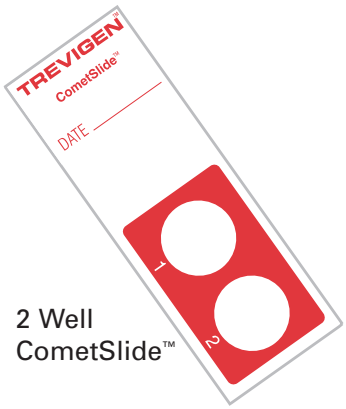
CometAssay™ Kits

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™ LM Agarose	15 ml	4250-050-02
200 mM EDTA; pH 10	12.5 ml	4250-050-04
SYBR® Green	5 µl	4250-050-05

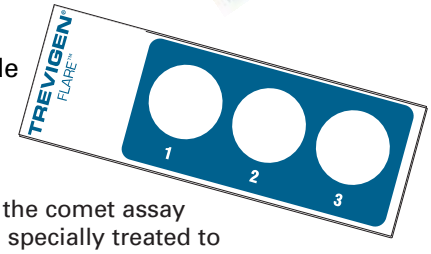
COMETSLIDES™



2 Well
CometSlide™

20 Well
CometSlide™

FLARE™ Slide



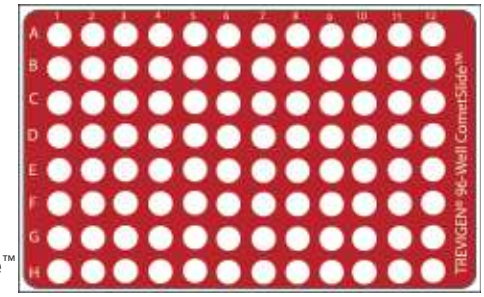
CometSlides™

CometSlides™ greatly simplify the comet assay by providing a sample surface specially treated to promote agarose adherence, and a hydrophobic barrier to allow treatment with one of Trevigen's DNA repair enzymes. Trevigen's three well FLARE™ Slide can also be interchanged with the CometSlide™. Simply add your cells to low melting point Comet LM Agarose, and pipet onto the slide.

CometSlides™

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 slide)	10 slides	4253-960-03
96 Well CometSlide™ (96 well slide)	20 slides	4253-02K-03
96 Well CometSlide™ (96 well slide)	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

96 Well
CometSlide™



CONTROL CELLS

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.

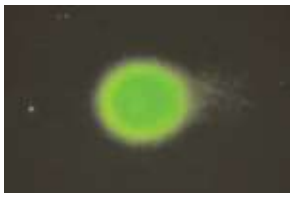
CometAssay™ Control Cells

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

CometAssay™ Control Cells Contents

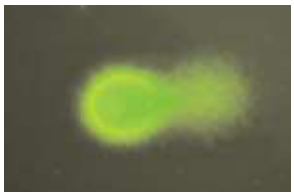
Component	Quantity	Catalog No.
Healthy Cells	500 µl	4256-010-CC0
Treated Cells - Level 1	500 µl	4256-010-CC1
Treated Cells - Level 2	500 µl	4256-010-CC2
Treated Cells - Level 3	500 µl	4256-010-CC3

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%



CC0 - Healthy Cells
0-10% DNA in Tail

CC1 - Treated Cells
11-30% DNA in Tail



CC2 - Treated Cells
31-50% DNA in Tail

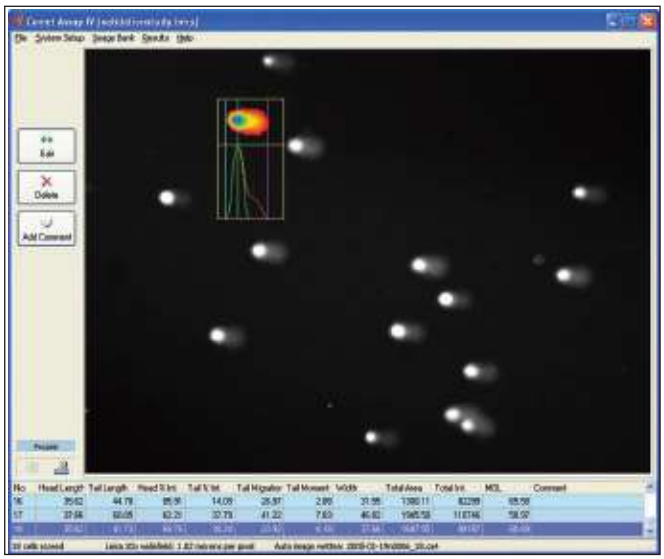
CC3 - Treated Cells
>50% DNA in Tail

DATA ANALYSIS

Comet Analysis

Electrophoreses cells once stained with a DNA specific fluorescent probe are typically visualized with a fluorescent microscope equipped with a camera. Cells are positioned with the bright cell nucleus or head to the left side of the camera view and the comet tail extending to the right. The fluorescence of the comet tail represents DNA that has migrated outside of the head region due to fragmentation. The fluorescent tail length increases as a function of DNA damage, but the maximum tail length is defined by the electrophoresis conditions. Fluorescent intensity continues to increase as more DNA migrates into the tail so optimization of electrophoresis conditions is critical.

Typical measurements are the percent DNA in the Tail (normalized to total cell DNA) and Tail Moment. Tail Moment is a damage measure combining the amount of DNA in the tail with the distance of migration (severity of damage). Commercial scoring systems provide analytic measures of the head and tail to quantify the degree of damage revealed by the comet assay. To allow for analysis of bright comet heads and dim comet tails, scoring systems differ based on the dynamic range of the camera and interpretation of the head position used to determine tail length and intensity measurements.



For Data Analysis packages see www.cometassay.com

CITATIONS

Recent Citations using Trevigen CometAssay™ Products –
Product descriptions and catalog numbers on pages 3 - 6

Replicative senescence induced by romo1-derived reactive oxygen species

Young Min Chung, Seung Baek Lee, Hyung Jung Kim, Seon Ho Park, Jung Jin Kim, Jin Sil Chung, and Young Do Yoo
J. Biol. Chem., Oct 2008; 10.1074/jbc.M805334200.

Experimental treatment of neuroblastoma using [131I]meta-iodobenzylguanidine and topotecan in combination

A G McCluskey, M Boyd, S L Pimlott, J W Babich, M N Gaze, and R J Mairs
Br. J. Radiol., Oct 2008; 81: S28 - S35.

Analysis of DNA breaks, DNA damage response, and apoptosis produced by high NaCl

Natalia I. Dmitrieva and Maurice B. Burg
Am J Physiol Renal Physiol, Oct 2008; 10.1152/ajprenal.90424.2008.

Molecular Regulation of DNA Damage-Induced Apoptosis in Neurons of Cerebral Cortex

Lee J. Martin, Zhiping Liu, Jacqueline Pipino, Barry Chestnut, and Melissa A. Landek
Cereb Cortex, Sep 2008; 10.1093/cercor/bhn167.

Interruption of the Ras/MEK/ERK signaling cascade enhances Chk1 inhibitor–induced DNA damage in vitro and in vivo in human multiple myeloma cells

Yun Dai, Shuang Chen, Xin-Yan Pei, Jorge A. Almenara, Lora B. Kramer, Charis A. Venditti, Paul Dent, and Steven Grant
Blood, Sep 2008; 112: 2439 - 2449.

Human Embryonic Stem Cells Have Enhanced Repair of Multiple Forms of DNA Damage

Scott Maynard, Anna Maria Swistowska, Jae Wan Lee, Ying Liu, Su-Ting Liu, Alexandre Bettencourt Da Cruz, Mahendra Rao, Nadja C. de Souza-Pinto, Xianmin Zeng, and Vilhelm A. Bohr
Stem Cells, Sep 2008; 26: 2266 - 2274.

Transcription-coupled DNA Double-Strand Breaks Are Mediated via the Nucleotide Excision Repair and the Mre11-Rad50-Nbs1 Complex

Josée Guirouilh-Barbat, Christophe Redon, and Yves Pommier
Mol. Biol. Cell, Sep 2008; 19: 3969 - 3981.

Inhibition of the p53 E3 Ligase HDM-2 Induces Apoptosis and DNA Damage – Independent p53 Phosphorylation in Mantle Cell Lymphoma

Richard J. Jones, Qing Chen, Peter M. Voorhees, Ken H. Young, Nathalie Bruey-Sedano, Dajun Yang, and Robert Z. Orlowski
Clin. Cancer Res., Sep 2008; 14: 5416 - 5425.

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."

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 LM Agarose.

Note: To ensure the cell concentration and dissociation are sufficient, a small aliquot can be diluted in PBS (instead of LM Agarose) 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.

FAQS

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™.

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. 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.

FAQS

8. 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.

9. 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.

10. 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.)

11. 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.

12. 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.

13. 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.

FAQS

14. 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.

15. 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.

16. 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.

17. 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.

18. 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?

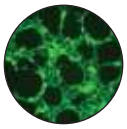
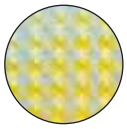
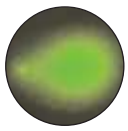
a) Yes, the volumes can be reduced to 50 µl.

19. 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.

Trevigen Cell Assays

TREVIGEN®

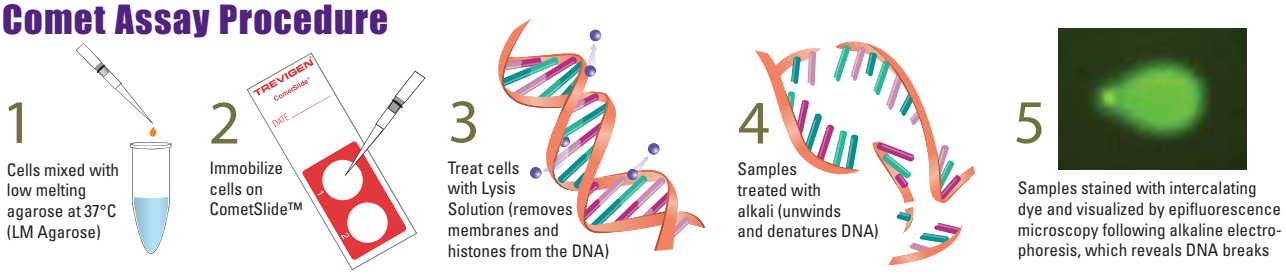
COMET ASSAY SERVICE

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

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. The scientists at TCA will collaborate with you to design screening studies employing the standardized CometAssay™ system and tailor it for your specific research requirements.

Comet Assay Procedure



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.

Coming Soon

TCA will soon be expanding its services to offer study design and compound screening services for:

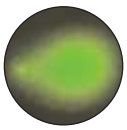
- PARP
- PARG
- Cell invasion
- Cell migration
- Tube formation

A Pharmacodynamic PAR assay will also be offered.

Remove this page and send it in to get your screening services started

Find the service at www.amsbio.com/customservices.aspx

Trevigen Cell Assays



COMET ASSAY SERVICE

Getting started is easy. Fax the completed form to ***+ 44(0)1235 820482*** *or email us at* ***info@amsbio.com*** *or complete the form online at* ***www.amsbio.com/customservices.aspx*** *with the information that we need in order to set up your screening service.*

Custom Quotation Form - Comet 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? *(eg CometAssay, Cell Proliferation Assay)*

How many samples do you have to screen?

How many replicates are required?

How many compounds and concentrations do you have to screen?

What types 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?

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

Remove this page and send it in to get your screening services started

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

Product	Size	Catalog No.
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
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

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 LM Agarose without base layers or pretreatment for three samples.