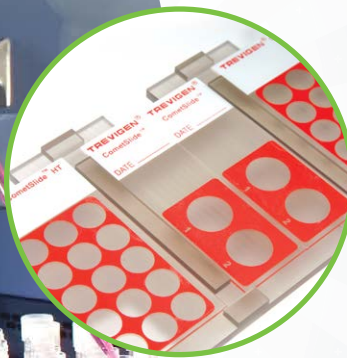


STANDARDIZED CometAssay® SYSTEM

TREVIGEN®



Instrumentation



Slides



Control Cells



CometAssay® Kits

CometAssay® PRODUCTS

CometAssay®

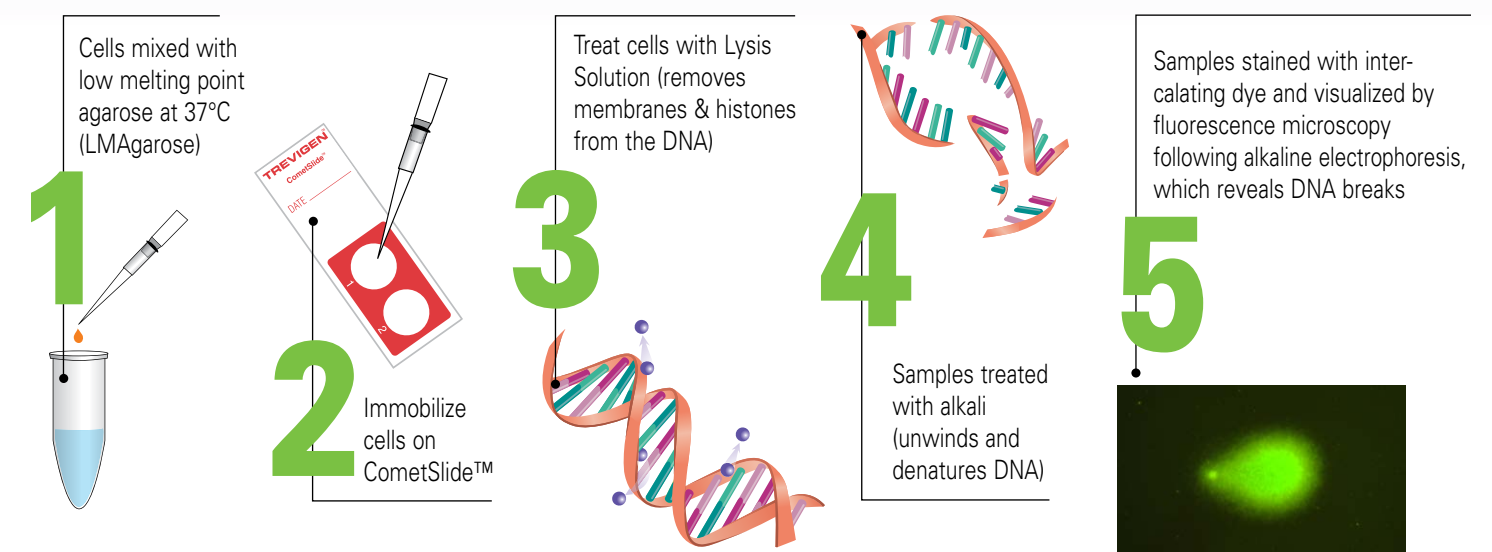
For the direct measurement of DNA Damage

The ability of chemical substances such as pesticides, metals, polycyclic aromatic hydrocarbons (PAHs), solvents, gasoline additives, etc. to induce mutations and DNA damage in prokaryotic and eukaryotic cells has created the need to test these chemicals for their genotoxic potential before being used as products, and therefore released into the environment. Conventional methods for evaluating genetic damage, including chromosomal aberration, micronucleus assays and their sister chromatid exchanges are time consuming, resource intensive and require proliferating cell populations. Hence, cost effective 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 in 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 impacting 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 and environmental biomonitoring. Used in conjunction with Trevigen's Electrophoresis Unit, the CometAssay® Control Cells, slides and reagents, measure double strand breaks (DSBs), single strand breaks (SSBs), alkali labile sites, oxidative DNA base damage, DNA crosslinking and DNA repair.

TREVIGEN®

1 800-TREVIGEN

www.trevigen.com
sales@trevigen.com



Complete Standardized System

CometAssay® Electrophoresis System

The standardized CometAssay® Electrophoresis System enables investigators to consistently optimize alkaline and neutral comet assays for highly reproducible results. It is used to standardize 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. Comet assay results can be variable depending on temperature, distance between electrodes, and buffer height. Trevigen, with funding from a Phase II SBIR grant**, has solved these problems and makes available a novel complete assay system which includes CometAssay® kits, CometSlides™, Alkaline and Neutral CometAssay® 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® Electrophoresis System. Products are available in a Starter Kit (4250-050-ESK) or as individual components listed in the table to the right. The 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® Alkaline Control Cells (4256-010-CC). Order Neutral CometAssay® Control Cells separately (4257-010-NC).



CometAssay® System

Instrumentation	Catalog No.
CometAssay® Electrophoresis System II	4250-050-ES
CometAssay® 2 Well ES Unit w/ Starter Kit	4250-050-ESK
CometAssay® 2 Well ES Unit w/ Starter Kit and Power Supply for North America	4250-050-ESK-PS1
CometAssay® 20 Well ES Unit w/ Starter Kit	4252-040-ESK
CometAssay® 20 Well ES Unit w/ Starter Kit and Power Supply for North America	4252-040-ESK-PS1
CometAssay® 96 Well ES Unit w/ Starter Kit	4253-096-ESK
CometAssay® 96 Well ES Unit w/ Starter Kit and Power Supply for North America	4253-096-ESK-PS1

Reagent Kits	Size	Catalog No.
CometAssay® Kit (25 X 2 well slides)	50 samples	4250-050-K
CometAssay® Kit (2 X 20 well slides)	40 samples	4252-040-K
CometAssay® Kit (1 X 96 well slide)	96 samples	4253-096-K
CometAssay® Silver Kit (25 X 2 well slides)	50 samples	4251-050-K
CometAssay® Silver Staining Kit components	200 samples	4254-200-K

Reagents	Size	Catalog No.
CometAssay® Lysis Solution	100 ml	4250-010-01
CometAssay® Lysis Solution	2 x 500 ml	4250-050-01
CometAssay® LMAgarose	15 ml	4250-050-02
CometAssay® LMAgarose	100 ml	4250-500-02

Control Cells	Size	Catalog No.
CometAssay® Alkaline Control Cells	10 assays	4256-010-CC
CometAssay® Neutral Control Cells	10 assays	4257-010-NC

Slides and Rack System	Size	Catalog No.
FLARE™ Slides (3 Well, 25 Slides)	25 slides	3950-075-02
FLARE™ Slides (3 Well, 100 Slides)	100 slides	3950-300-02
CometSlide™ (2 Well, 2 Slides)	2 slides	4250-004-03
CometSlides™ (2 Well, 25 Slides)	25 each	4250-050-03
CometSlides™ (2 Well, 100 Slides)	100 slides	4250-200-03
CometSlide™ Rack System	each	4252-040-02
CometSlide™ (96 Well)	1 slide	4253-096-03
CometAssay® HT Slides (20 Well, 2 Slides)	2 slides	4252-040-01
CometAssay® HT (20 Well, 10 Slides)	10 slides	4252-200-01
CometAssay® HT (20 Well, 25 Slides)	25 slides	4252-500-01
CometAssay® HT Slides (20 Well, 100 Slides)	100 slides	4252-02K-01

FLARE™ Assay Kits and Modules	Size	Catalog No.
FLARE™ Sample Prep	75 tests	3950-075-SP
Fpg FLARE™ Assay Kit	75 samples	4040-100-FK
Fpg FLARE™ Module	100 samples	4040-100-FM
E. coli Endonuclease III FLARE™ Kit	75 samples	4045-01K-FK
E. coli Endonuclease III FLARE™ Module	100 samples	4045-01K-FM
hOGG1 FLARE™ Assay Kit	75 samples	4130-100-FK
hOGG1 FLARE™ Module	100 samples	4130-100-FM

CometChip® System	Size	Catalog No.
CometChip® Starter Kit	96 samples	4260-096-CSK
CometChip® Kit	96 samples	4260-096-K
96-Well CometChip® System	1 each	4260-096-CS
CometChip®	30 micron pores	4260-096-01

*PS = Power Supply

**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.

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 consistent DNA migration. A constant buffer temperature is maintained using an underlying ice pack to cool the slide platform and buffer chamber. 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.

Kits

CometAssay® Kits

Trevigen's CometAssay® provides reagents and our exclusive CometSlide™ for the rapid analysis of DNA fragmentation associated with DNA damage. Following lysis, the unwound, relaxed DNA is able to migrate out of the cell during alkaline or neutral electrophoresis and can be visualized using SYBR® Gold nucleic acid gel stain. Cells that have accumulated DNA damage appear as fluorescent comets with tails of DNA fragmentation or



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 variability between individual users and different labs

CometAssay® Electrophoresis Unit

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

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.

CometAssay® Kits

Description	Size	Catalog No.
CometAssay® Kit (2 well)	50 samples	4250-050-K
CometAssay® HT Kit (20 well)	40 samples	4252-040-K
CometAssay® Kit (96 well)	96 samples	4253-096-K
CometAssay® Silver Kit (2 well)	50 samples	4251-050-K
CometAssay® Silver Straining Kit Components	50 samples	4254-200-K

CometAssay® Reagents

Description	Size	Catalog No.
CometAssay® Lysis Solution	100 ml	4250-010-01
CometAssay® Lysis Solution	2 x 500 ml	4250-050-01
CometAssay® LMAgarose	15 ml	4250-050-02
CometAssay® LMAgarose	100 ml	4250-500-02

CometSlides™

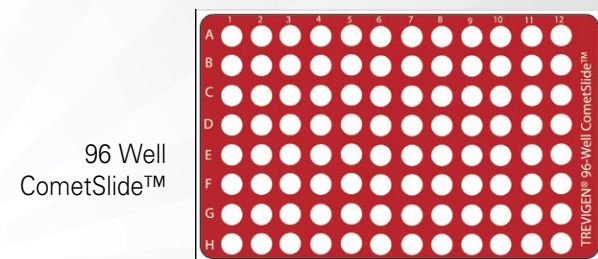


CometSlides™

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. The three well FLARE™ Slide can also be interchanged with the CometSlide™. Simply add your cells to the low melting point Comet LMAgarose, and pipet onto the slide.

CometSlides™

Description	Size	Catalog No.
CometSlide™ (2 Well, 2 Slides)	2 slides	4250-004-03
CometSlides™ (2 Well, 25 Slides)	25 slides	4250-050-03
CometSlides™ (2 Well, 100 Slides)	100 slides	4250-200-03
CometSlide™ (96 Well)	1 slides	4253-096-03
CometAssay® HT (20 Well, 2 Slides)	1 slides	4252-040-01
CometAssay® HT (20 Well, 10 Slides)	10 slides	4252-200-01
CometAssay® HT (20 Well, 25 Slides)	25 slides	4252-500-01
CometAssay® HT Slides (20 Well, 100 Slides)	100 slides	4252-02K-01
FLARE™ Slides (3 Well, 25 Slides)	25 slides	3950-075-02
FLARE™ Slides (3 Well, 100 Slides)	100 slides	3950-300-02
CometSlide™ Rack System	each	4252-040-02



Control Cells

CometAssay® Control Cells

For the standardization of comet assays

Trevigen's CometAssay® Alkaline and Neutral Control Cells are sets 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 sets 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. Healthy control cell populations (CC0 or NCO) were treated under various conditions to increase the amount of damage in the corresponding populations. These cryopreserved control cells are designed to act as controls to standardize and compare electrophoresis methods between individual users and laboratories.



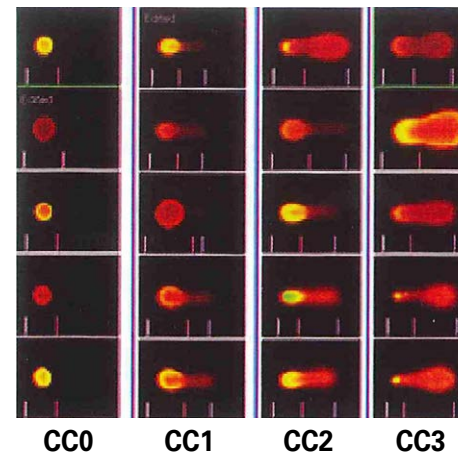
CometAssay® Control Cells

Description	Size	Catalog No.
CometAssay® Alkaline Control Cells	1 Set (10 Assays)	4256-010-CC
CometAssay® Neutral Control Cells	1 Set (10 Assays)	4257-010-NC

CometAssay® Control Cells (Alkaline)

Component	Quantity	% DNA in Tail	Catalog No.
Healthy Cells	500 µl	5.76%	4256-010-CC0
Treated Cells - Level 1	500 µl	28.37%	4256-010-CC1
Treated Cells - Level 2	500 µl	39.74%	4256-010-CC2
Treated Cells - Level 3	500 µl	56.80%	4256-010-CC3

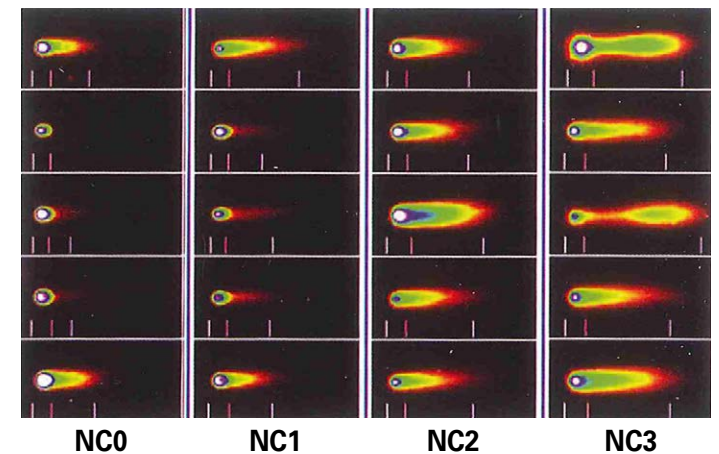
Typical Alkaline CometAssay® Results



CometAssay® Control Cells (Neutral)

Component	Quantity	Tail Moment	Catalog No.
Healthy Cells	500 µl	0.68	4257-010-NC0
Treated Cells - Level 1	500 µl	4.32	4257-010-NC1
Treated Cells - Level 2	500 µl	15.71	4257-010-NC2
Treated Cells - Level 3	500 µl	25.73	4257-010-NC3

Typical Neutral CometAssay® Results

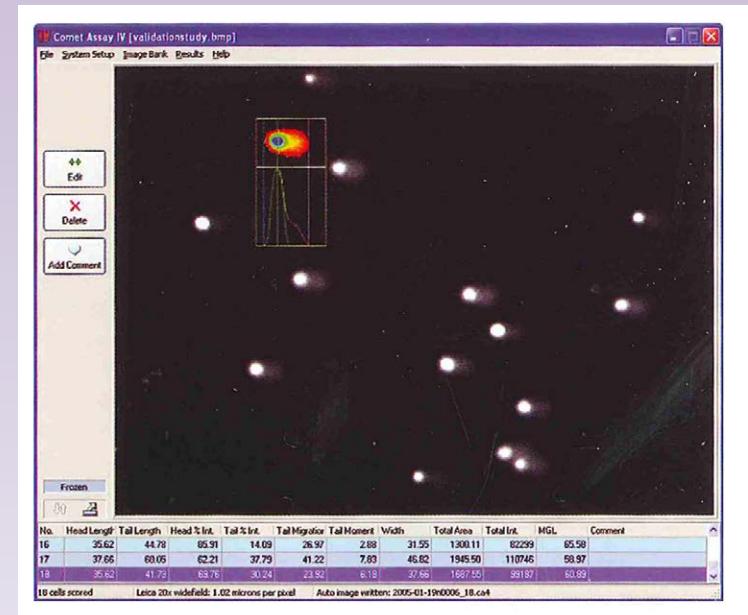


Data Analysis

Comet Analysis

Electrophoresed 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, but easy if a CometAssay® ES unit is used.

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.

Coming Soon



96-Well CometChip® System



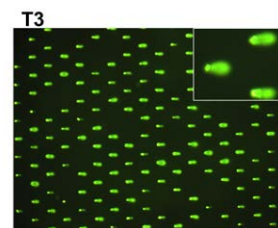
Designed for CometAssay® reagents and Electrophoresis System

CometChip® for High Throughput CometAssay®

The 96-Well CometChip® System is a high-throughput platform to simultaneously treat and measure DNA damage induced by different treatments, or among different cell types on a single slide using the comet assay. The CometChip® is a consumable consisting of specifically sized micron pores patterned into agarose. 96 separate wells are created by inserting the CometChip® into a reusable magnetically-sealed cassette suitable for tissue culture incubators. Cells added to each well are captured by gravity into micropores and excess cells are aspirated, leaving an array of non-overlapping cells. Multiple experimental conditions are performed in parallel by the addition of different chemicals to respective wells. Once treatment is complete the CometChip® is removed from the cassette and processed using standard alkaline comet conditions and imaging systems.

CometChip® Products

Description	Size	Catalog No.
CometChip® (30 micron)	each	4260-096-01
CometChip® Kit	96 samples	4260-096-K
96-Well CometChip® System	1 each	4260-096-CS
CometChip® Starter Kit	96 samples	4260-096-CSK
CometChip® Electrophoresis Starter Kit	1 each	4260-096-ESK



Sample 4X Macrowell Image

Related Products

Trevigen's unique FLARE™ (Fragment Length Analysis using Repair Enzymes) Assays provide the ability to characterize DNA damage in single cells using a variety of DNA repair enzymes in conjunction with Trevigen's CometAssay® Electrophoresis System. 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. Following electrophoresis, an apparent increase in damage as a result of enzyme addition is indicative of unrepaired but recognized molecular lesions within the DNA.

Researchers who purchased CometAssay® products also purchased these.

FLARE™ Assay Kits and Modules

Catalog #	Product Name	Size
3950-075-SP	FLARE™ Sample Prep	75 tests
4040-100-FK	Fpg FLARE™ Assay Kit	75 samples
4040-100-FM	Fpg FLARE™ Module	100 samples
4045-01K-FK	E. coli Endonuclease III FLARE™ Kit	75 samples
4045-01K-FM	E. coli Endonuclease III FLARE™ Module	100 samples
4130-100-FK	hOGG1 FLARE™ Assay Kit	75 samples
4130-100-FM	hOGG1 FLARE™ Module	100 samples

Citations

c-MVC suppresses BIN1 to release Poly(ADP-Ribose) Polymerase1: A mechanism by which cancer cells acquire cisplatin resistance Neutral CometAssay®

Siovenie Pyndiah, Satoshi Tanida, Kazi M. Ahmed, Erica K. Cassimere, Chungyoul Choe, and Daitoku Sakamuro
Sci. Signal., Mar 2011; 4: ra19.

The Set2-RPB1-interaction domain of human REC05 is important for transcription-associated genome stability Neutral CometAssay®

Min Li, Xiaohua Xu, and Yilun Liu
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Glycogen synthase kinase 3β inhibition enhances repair of DNA double-strand breaks in irradiated hippocampal neurons Neutral CometAssay®

Eddy S. Yang, Somaira Newsheen, Tong Wang, Dinesh K. Thotala, and Fen Xia
Neuro Oncology, Mar 2011; 10.1093/neuonc/nor016

Peroxiredoxin II restrains DNA damage-induced death in cancer cells by positively regulating JNK-dependent DNA repair Neutral CometAssay®

Kyung Wha Lee, 000 Jae Lee, Joo Young Lee, Dong Hoon Kang, Jongbum Kwon, and Sang Won Kang
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Poly(ADP-ribose) polymerase and XPF-ERCC1 participate in distinct pathways for the repair of topoisomerase I-induced DNA damage in mammalian cells Neutral CometAssay®

Yong-Wei Zhang, Marie Regairaz, Jennifer A. Seiler, Keli K. Agama, James H. Doroshov, and Yves Pommier
Nucleic Acids Res., Jan 2011; 10.1093/nar/gkq1304

CDDO-Imidazolide induces DNA damage, G2/M arrest and apoptosis in BRCA 1-mutated breast cancer cells

Alkaline CometAssay®

Eun-Hee Kim, Chu-Xia Deng, Michael B. Sporn, and Karen T. Liby
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Neutral CometAssay®

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Modulating endogenous NQO1 levels identifies key regulatory mechanisms of action of β-Lapachone for pancreatic cancer therapy Alkaline CometAssay®

Long Shan Li, Erik A. Bey, Ying Dong, Jieru Meng, Biswanath Patra, Jingsheng Van, Xian-Jin Xie, Rolf A. Brekken, Carlton C. Barnette, William G. Bornmann, Jinming Gao, and David A. Boothman
Clin. Cancer Res., Jan 2011; 17: 275 -285

Epstein-Barr virus DNase (BGLF5) induces genomic instability in human epithelial cells

Alkaline CometAssay®

Chung-Chun Wu, Ming-Tsan Liu, Yu-Ting Chang, Chih-Yeu Fang, Sheng-Ping Chou, Hsin-Wei Liao, Kuan-Lin Kuo, shih-Lung Hsu, Yi-Ren Chen, Pei-Wen Wang, Yu-Lian Chen, Hsin-Ying Chuang, Chia-Huei Lee, Ming Chen, Wun-Shaing Wayne Chang, and Jen-Yang Chen
Nucleic Acids Res., Apr 2010; 38: 1932 -1949

FAQS

1. Will any commercial horizontal gel electrophoresis device work for electrophoresis?

Comet assay variability led to the development of our new CometAssay® Electrophoresis System. Conventional slab gel electrophoresis chambers are not designed to eliminate all known causes of comet assay variability. They can be used, but they require optimization by the user to achieve consistent results. We recommend using Trevigen's CometAssay® Electrophoresis System which addresses these issues and for highly reproducible results.

2. Why is LMAgarose recommended?

The agarose used must be a liquid at 37° C. Use of gel electrophoresis agarose is not recommended due to the risk of introducing DNA damage at high temperatures.

3. Why is there loss of agarose disks from the CometSlide™?

CometSlides™ are specially treated in order to enhance agarose binding during alkaline treatment. Special attention needs to be paid during spreading of LMAgarose on the slides. Generally, place 50 ul LMAgarose on the center of each well (2 well slides) and then gently spread the agar using the side of a pipette tip. You don't want a mound of agar in the center, because the adhesion area needs to be in contact with the agar over the entire well. Be careful not to get any LMAgarose on the red or blue masking for when it dries, it increases the chances of the agar coming off or folding over.

- All processing of the slides needs to be done very gently. Place the slides into solutions; never pour or decant buffer onto CometSlides™.
- For more detailed information please see: www.trevigen.com.

4. How often do you recommend using CometAssay® Control Cells?

Use of control cells in each and every electrophoresis run is recommended as a best practice to generate high quality CometAssay® data. Please see: Kumaravel et al., Comet Assay measurements: a perspective. Cell Bio Toxicol (2009) 25:53-64.

5. The high throughput comet slide (CometSlide™ HT 4252-200-01) has 20 sample wells; is it possible to re-use the slide if not all wells were utilized?

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

6. Is there a convenient step to stop the CometAssay® and resume the next day due to time constraints?

It should be possible to cryopreserve your samples on day one, run the assay on day two; dry the LMAgarose disks

containing the electrophoresed nucleoids down, and store overnight with staining on day three.

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

We do not recommend using whole blood with the CometAssay® because sample preparation is critical and hemoglobin could damage DNA. It's important to note that red blood cells (major blood component) do not have a nucleus (i.e. genomic DNA) and therefore are not suitable for use with CometAssay®. Rather, assay of isolated leukocytes is preferred.

8. What image analysis tools are available for scoring comets?

PC software: A free 3--day trial of Comet Assay IV, a commercial software system designed specifically for the comet assay, can be downloaded from <http://www.scorecomets.com>. Comet Assay IV includes all major measurement parameters and is the most advanced system available on the market today. Features include point-and-click scoring and options for regulatory compliance.

Mac software: NIH Image, a free general purpose image analysis tool, is available from <http://rsb.info.nih.gov/nih-image>. This software can be used along with custom macros specifically designed to give comet tail moment. A useful macro for calculating the tail moment from saved digital images has been provided by Professor Herbert M. Geller, Ph.D.

If you need assistance regarding analysis of large sample sets, please contact us!

9. How long can a CometSlide™ (4250-050-03) be stored?

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

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

Carefully prepared cell suspensions from tissues can be used with the CometAssay®.

Protocol Guideline

- Place a small piece of tissue in 1-2 ml of cold HBSS containing 20 mM EDTA.
- Mince the tissue into fine pieces and allow settling.
- Remove 5-10 ml of the supernatant (cell suspension) and combine with 75 ml molten LMAgarose. 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.

For more FAQS, please visit www.trevigen.com

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 by 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. "

About Us

Trevigen, Inc is an innovative biotechnology company focused on the development of products and technologies for cell behavior, including stem cell/ regenerative medicine and cancer research. Trevigen has core technologies in protein purification, cell biology and DNA damage and repair. The company is the recipient of several NIH SBIR grants from the National Institute of Health, concerning technology development for the analysis of DNA Damage, and an SBIR Contract for the development of an in vivo co-culture breast cancer model. The Trevigen Research and Development team has a number of thought leaders in the fast growing 3-D Cell Culture research field, who have helped the company become a pioneer in the industry by developing some of the first commercially available products for 3-D Culture research.

Comet Assay Service

At your request, the scientists at TCA (Trevigen Cell Assays- our contract research division) will collaborate with you to design screening studies employing the CometAssay® tailored for your specific needs. Contact us and request a quote.

TREVIGEN®

www.trevigen.com • sales@trevigen.com • 1 800-TREVIGEN

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1 800-TREVIGEN

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sales@trevigen.com