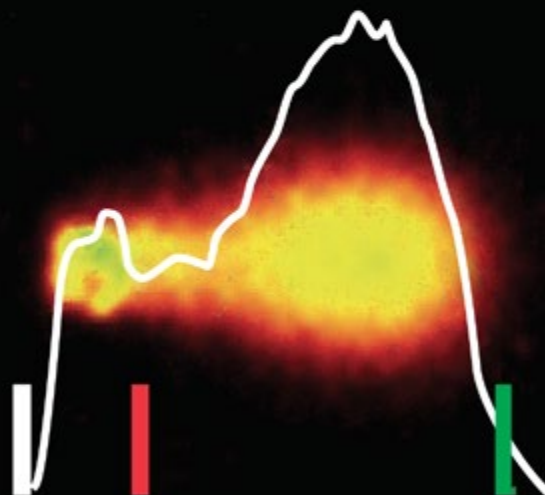


biotechne®

CometAssay® System

Standardized System for DNA Damage Analysis



R&D SYSTEMS
a biotechne® brand

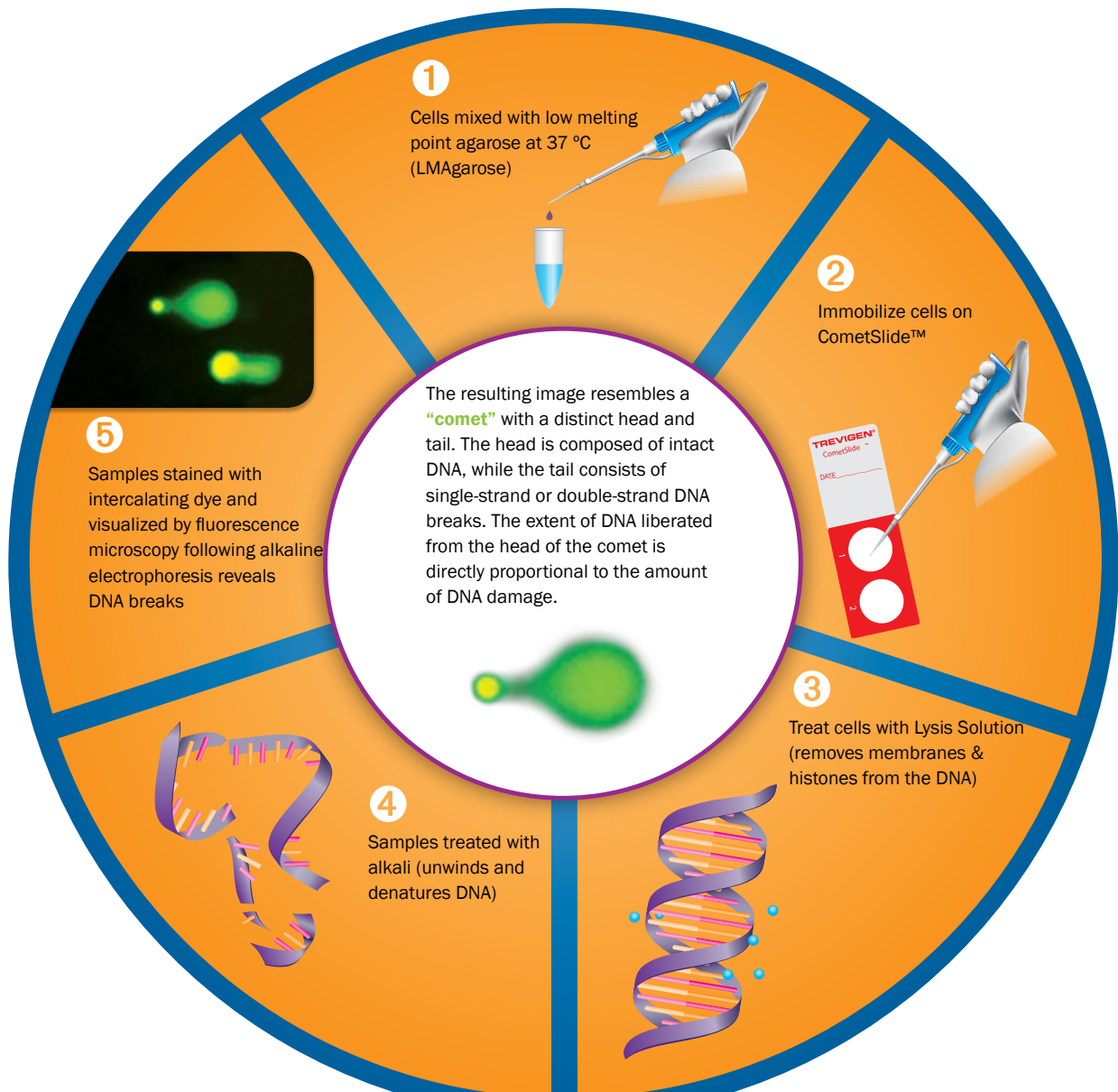
Trevigen®

Introduction to CometAssay®

The comet assay provides high sensitivity in the detection of DNA breaks in single cells, but changes in method or reagents can result in variation between users and laboratories. The CometAssay Standardized System provides qualified reagents, control cells, slides, instrumentation and software for highly reproducible results in low and high throughput testing.

Method

The comet assay is a sensitive technique for quantifying DNA damage and repair from *in vivo* and *in vitro* samples of eukaryotic and plant cells. This is the only technique that directly measures DNA damage in individual cells and thus has rapidly gained importance in the fields of genetic toxicology and human biomonitoring.



CometAssay® Benefits

- Qualified reagents and enzymes increase assay sensitivity
- Control cell standards to compare runs
- Treated slides save time and costs with multiple formats
- Optimized instrumentation minimize electrophoresis variability
- Automated analysis software save time and minimize bias
- Tech support tutorials and webinars
- Contract services expertise to design studies for your specific needs

CometAssay® Products

Starter Kits

Starter Kits include a CometAssay Reagent Kit, Alkaline Control Cells and the CometAssay Electrophoresis System with optional Power Supply. Just select the slide format (2, 20 or 96 well slides).

Product	Catalog #
Comet Assay 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	4250-040-ESK-PS
CometAssay 20 Well ES Unit w/ Starter Kit	4252-040-ESK
CometAssay 20 Well ES Unit w/ Starter Kit and Power Supply	4252-040-ESK
CometAssay 96 Well ES Unit w/ Starter Kit	4253-096-ESK
CometAssay 96 Well ES Unit w/ Starter Kit and Power Supply	4253-096-ESK-PS

Software

Product	Amount	Catalog #
Comet Analysis Software	1 license	4260-000-CS

Specifications

- Designed to work under Windows 10, 8/8.1 and 7
- Compatible with 4X and 10X images
- Compatible with TIFF, JPEG and PNG image formats
- Software does not provide capabilities for direct control of camera for image capture. Images must be captured outside of software

CometAssay® Reagents

Product	Amount	Catalog #
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
200 mM EDTA, pH 10	12.5 ml	4250-050-04

CometAssay® Reagent Kits

Product	Amount	Catalog #
CometAssay Kit (25 x 2 Well Slides)	50 samples	4250-050-K
CometAssay Kit (2 x 20 Well Slides)	40 samples	4252-040-K
Comet Assay Kit (1 x 96 Well Slide)	96 samples	4253-096-K
CometAssay Silver Kit (2 Well Slides)	50 samples	4251-050-K
CometAssay Silver Staining Components	200 samples	4254-200-K

Related Products

CometChip® Electrophoresis Starter Kit (Catalog # 4260-096-ESK)		
Component	Amount	Catalog #
CometChip Starter Kit	1 each	4260-096-CSK
CometAssay Electrophoresis System II	1 system	4250-050-ES

CometChip® Starter Kit (Catalog # 4260-096-CSK)		
Component	Amount	Catalog #
CometChip Reagent Kit	1 each	4260-096-K
96-Well CometChip System	1 system	4260-096-CS

Slides and Rack System

Product	Amount	Catalog #
FLARE Slide (3 Well)	25 Slides	3950-075-02
FLARE Slide (3 Well)	100 Slides	3950-300-02
CometSlide 2 Well	2 Slides	4250-004-03
CometSlide 2 Well	25 Slides	4250-050-03
CometSlide 2 Well	100 Slides	4250-200-03
CometSlide 20 Well	2 Slides	4252-040-01
CometSlide 20 Well	10 Slides	4252-200-01
CometSlide 20 Well	25 Slides	4252-500-01
CometSlide 20 Well	100 Slides	4252-02K-01
CometSlide 96 Well	1 Slide	4253-096-03
CometSlide Rack System	1 each	4252-040-02

FLARE™ Assay Kits

Product	Amount	Catalog #
FPG FLARE Assay Kit	75 samples	4040-100-FK
hOGG1 FLARE Assay Kit	75 samples	4130-100-FK

FLARE™ Assay Modules and Sample Prep

Product	Amount	Catalog #
FLARE Sample Prep	75 samples	3950-075-SP
FPG FLARE Module	100 samples	4040-100-FM
hOGG1 FLARE Module	100 samples	4130-100-FM
Hydrophobic Coverslips	100 each	4867-100

Control Cells

Product	Amount	Catalog #
CometAssay Alkaline Control Cells	1 set (10 Assays)	4256-010-CC
CometAssay Neutral Control Cells	1 set (10 Assays)	4257-010-NC

Contract Services (Catalog # 6000-TCA)

Format	Protocol	Project Example
20-Well CometAssay	4252-040-K	20-100 sample wells per run
96-Well CometChip	4260-096-CSK	96-288 sample wells per run

CometChip® Reagent Kit (Catalog # 4260-096-K)		
Component	Amount	Catalog #
CometChip, 30 micron	1 each	4260-096-01
Lysis Solution	100 ml	4250-010-01
Comet LMAgarose	15 ml	4250-050-02

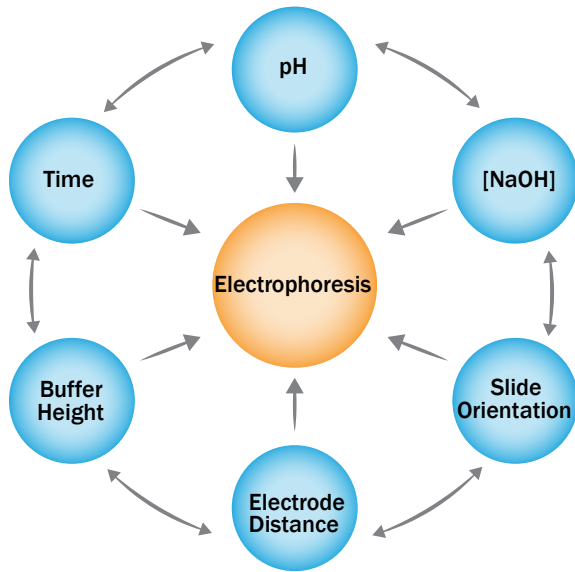
Instrumentation

CometAssay® Electrophoresis System II

Optimized Alkaline Electrophoresis of CometSlides™

Unique Features

- Overcomes variations by placing an acrylic overlay on top of an elevated slide tray to maintain optimal buffer height for consistent DNA migration
- Maintains constant buffer temperature with 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 electrophoretic field
- Starter Kits include CometAssay Reagent Kit and Alkaline Control Cells optimized for use with the CometAssay Electrophoresis System and Power Supply



Software

Comet Analysis Software

Comets from Well Images Automatically Located and Scored in Seconds

Unique Features

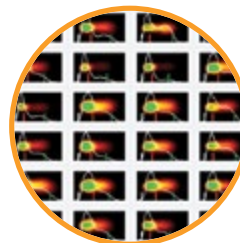
- Use existing microscope and camera for image capture
- Uses 4X and 10X images
- Step-by-step Instructions—with Comet Wizard
- Drag-and-drop images into slide library
- Automatic comet finding and Scoring
- Instant and unbiased analysis
- Affordable at single flat rate with no service fee

Automatic Comet Finding



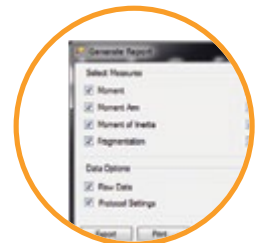
✓ Review from image

Automatic Scoring



✓ Edit based on profile

Generate Reports



✓ User options



Training video available | trevigen.com (search 4260-000-CS)

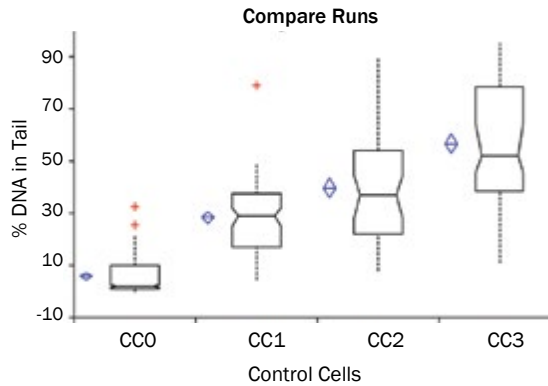
Control Cells

CometAssay® Control Cells

Viable cell suspensions with incremental increases in DNA damage

Unique Features

- For the standardization of comet assays between individual users
- Provides a known range of DNA damage for comparison with four statistically distinct populations
- Two sets of control cells available when performing either Alkaline or Neutral Comet Assay
- Cell populations remain stable in liquid nitrogen for long term storage
- Standardized with CometAssay Electrophoresis System II



Cryopreserved
Store long term



Treated Slides

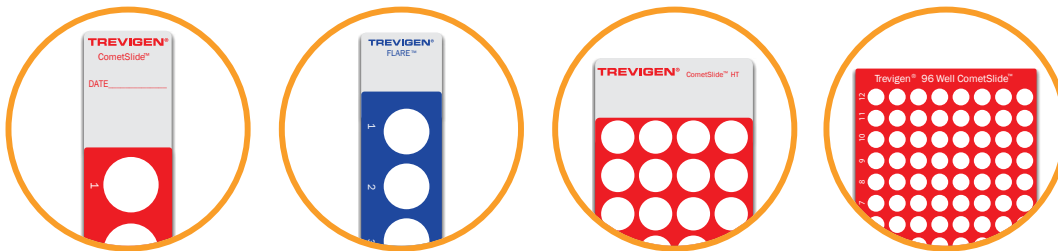
CometSlides™ and Rack System

Simply add your cells to Comet LMAgarose and pipet onto the slide

Unique Features

- Specially treated to promote adherence of low melt agarose during alkaline steps
- Hydrophobic barrier for easy application of cells, staining solution, and enzyme treatment to improve assay sensitivity
- The 2 and 3-well slides are interchangeable
- Easily scalable based on slide format to save time and reagents when processing larger sample numbers
- Rack System accommodates 2, 3 or 20 well slides

Multiple Formats Save Time & Costs



Reagent Kits

CometAssay® Reagent Kit

Just select slide format to minimize number of runs

Kit Includes

- Lysis solution
- LMAGarose
- 200 mM EDTA, pH 10
- CometSlides
- Detailed Instructions



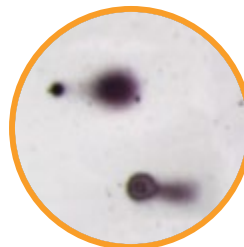
Fluorescent Staining

CometAssay® Silver Kit

Allows visualization by standard light microscopy

Unique Features

- Permanent staining for archiving samples
- Optimized for CometSlide staining
- Includes Silver Staining Components and CometAssay Kit (25 x 2 Well Slides)
- Silver Staining Components available separately



Silver Staining

Reagent Kits with Enzymes

FLARE™ Assay Kits

Fragment Length Analysis using Repair Enzymes in the CometAssay

Unique Features

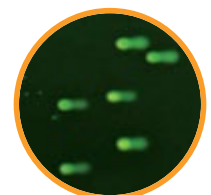
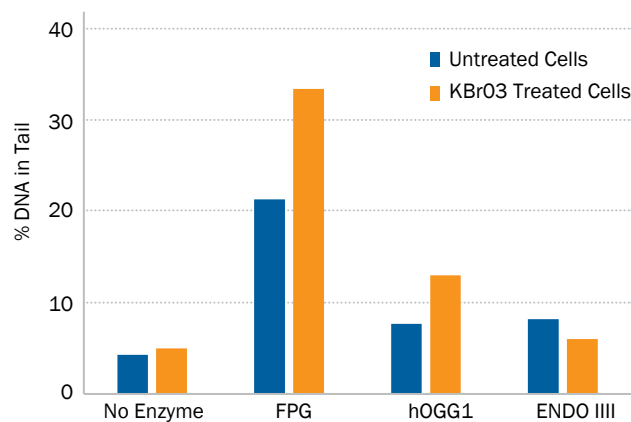
DNA Repair Enzymes increase CometAssay sensitivity

- Qualified enzymes and buffers
- Designed to work with CometAssay System

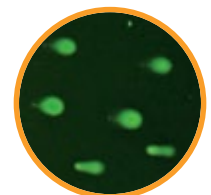
- Kits include FLARE Sample Prep (Slides & Reagents) and FLARE Module (Enzyme & Buffers)
- FLARE Sample Prep and Modules are available separately

Increase in damage with enzyme addition is indicative of unrepaired but recognized lesions within the DNA.

- 1 Harvest cells and immobilize in LMAGarose on FLARE Slide
- 2 After lysis, incubate samples with lesion specific DNA Repair Enzymes
- 3 Perform alkaline electrophoresis



No Enzyme - 5 µg/ml MMS



FPG - 5 µg/ml MMS

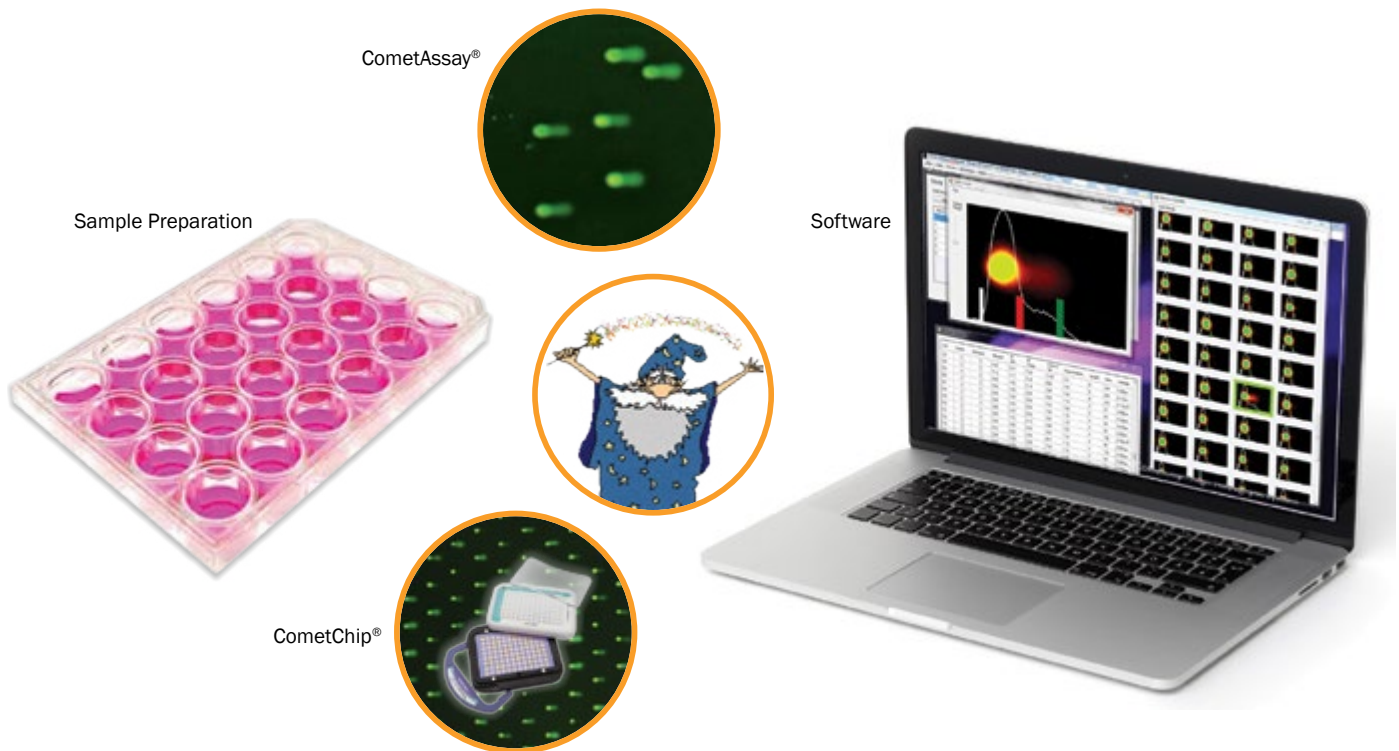
Contract Services

Trevigen® Cell Assays (TCA) specializes in tailoring and conducting assays for lead compounds and genotoxic screening based on DNA damage and repair using Trevigen products (CometAssay®, CometChip®, Pharmacodynamic Assays, and PARP/PARG Assays) as well as cancer cell behavior (Cultrex® Invasion, Migration and Tube Formation Assays).

Comet Assay Service

Simply complete the online form for Catalog # 6000-TCA Comet Assay and select the format to receive a quote.

- CometAssay is the traditional single cell gel electrophoresis protocol to quantitate DNA damage by immobilizing a mixture of low melt agarose and cells onto a treated glass slide.
- CometChip Platform is new and designed for high-throughput providing high density data with minimal overlaps by capturing cells into an array of micropores.
- Samples may be prepared by customer or at Trevigen.
- Comet Analysis only for slides prepared by customer is also available.



Frequently Asked Questions

Will any commercial horizontal gel electrophoresis device work for electrophoresis?

Electrophoresis conditions described for FLARE Assay, CometAssay and CometChip Kits were optimized using Trevigen's CometAssay Electrophoresis System II. Conventional slab gel electrophoresis chambers are not designed to eliminate known causes of comet assay variability (alkaline pH, buffer height, temperature, slide orientation). They can be used, but they require optimization by the user to achieve consistent results.

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.

Is it possible to reuse CometSlides™?

FLARE and CometSlides contain a special coating for binding the LMAgarose, which can be compromised if washed for re-use.

How should the CometAssay® Control Cells be stored?

The CometAssay Control Cells should be stored in liquid nitrogen. To avoid the accumulation of damage due to freeze thaw, the CometAssay Control Cells should be thawed and frozen in working aliquots

Is it possible to use formalin-fixed tissues or cells when performing the comet assay?

The comet assay requires viable cells and care when preparing the single cell suspension.

Why is there DNA damage in control cell samples isolated from blood?

The addition of DMSO to the Lysis Solution (10% final) is recommended for samples containing heme, such as blood cells or tissue samples. DMSO acts as a scavenger to prevent heme induced DNA damage.

Is it possible to store sample slides after embedding cells in LMAgarose?

Once the cells have been embedded in the LMAgarose, the comet assay protocol needs to be completed. However, it is very convenient to leave the sample slides overnight at 4 °C in Lysis Solution when performing multiple treatments at multiple times.

Does the agarose need to completely dry after electrophoresis?

It is important to completely dry the agarose disk to bring the cells into a single plane for viewing.

Is there a particular % tail intensity to indicate cytotoxicity rather than DNA damage?

It has been shown that cell populations with high levels of damage ~80–90% are capable of DNA Repair and within 1–2 hr return to basal levels. The best recommendation is to monitor the viability of the cell population. The comet assay should be performed with cell populations having at least 75% viability following treatment.

Is the Lysis Solution suitable for both the alkaline and neutral comet assays?

The Lysis Solution is pH 10 and used for cell lysis in both the alkaline and neutral comet assays.

Why is there only diffusion of the head and no comet tails in my positive controls?

If not using Trevigen's CometAssay Electrophoresis System II, maintaining a minimal buffer above the slides is critical when using an alkaline buffer. As the buffer height increases, DNA migration of the tail decreases. Trevigen offers Alkaline Control Cells (4256-010-CC) with known levels of DNA damage which can be used to optimize electrophoresis conditions.

Which comet assay is the most sensitive?

The alkaline comet assay is the most sensitive since the DNA is denatured allowing the detection of both single-strand and double-strand cuts and abasic lesions.

After spreading the agarose cell mixture on the slide, can we leave it overnight?

Once the agarose has solidified, the slides should be immediately placed in Lysis Solution at 4 °C. The cells are still viable and capable of DNA repair until lysis.

Why aren't the comets staining with silver?

Fixation is performed immediately before staining on dried wells. After 20 minute fixation it is critical to rinse well with water to remove all the residual acetic acid or else staining will not occur.

What Customers are Saying

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.

Larry Gladnick
Pharmaceutical Scientist

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.**

Avinash M. Tope, Ph.D.
Principal Investigator,
Kentucky State University

We were introduced to **Trevigen's scoring software** at a vendor expo and were very eager to try it with our samples and found that time to score a complete set of 96 samples from beginning to final spreadsheets and graphs was significantly reduced. What had been taking 2–4 days now took less than 1 full day! **Not only is scoring faster, but Trevigen's customer service has been very responsive to our specific needs and goes the extra mile** to ensure our satisfaction with their products.

Karen Cooper, Ph.D.
University of New Mexico, Pharmaceutical Sciences

References using CometAssay® Technology

HT-COMET: a novel automated approach for high throughput assessment of human sperm chromatin quality Océane Albert, Wolfgang E. Reintsch, Peter Chan, and Bernard Robaire. Hum. Reprod., May 2016; 31: 938 - 946

Interactive Genotoxicity Induced by Environmentally Relevant Concentrations of Benzo(a)Pyrene Metabolites and Arsenite in Mouse Thymus Cells. Huan Xu, Fredine T. Lauer, Ke Jian Liu, Laurie G. Hudson, and Scott W. Burchiel. Toxicol. Sci., Aug 2016; 154: 153-161

Gata6 promotes hair follicle progenitor cell renewal by genome maintenance during proliferation. Alex B. Wang, Ying V. Zhang, and Tudorita Tumber. EMBO J., Jan 2017; 36: 61-78

Ubiquitylation of Ku80 by RNF126 Promotes Completion of Nonhomologous End Joining-Mediated DNA Repair. Noriko Ishida, Tadashi Nakagawa, Shun-Ichiro Iemura, Akira Yasui, Hiroki Shima, Yasutake Katoh, Yuko Nagasawa, Toru Natsume, Kazuhiko Igarashi, and Keiko Nakayama. Mole. Cell. Biol., February 2017; 37: e00347-16

Induction of cytotoxic and genotoxic damage following exposure of V79 cells to cadmium chloride. Jillian Gobrecht, Claire McDyre, James Comotto, and Mindy Reynolds. Mutation Research, March 2017; 816-817: 12-17

Withaferin-A kills cancer cells with and without telomerase: chemical, computational and experimental evidences. Yue Yu, Shashank P. Katiyar, Durai Sundar, Zeenia Kaul, Elijiro Miyako, Zhenya Zhang, Sunil C. Kaul, Roger R. Reddel and Renu Wadhwa. Cell Death & Disease, April 2017; 8: e2755

CYT-Rx20 inhibits ovarian cancer cells in vitro and in vivo through oxidative stress-induced DNA damage and cell apoptosis. Yen Y. Wang, Yuk K. Chen, Stephen C. Hu, Ya L. Hsu, Chun H. Tsai, Tsung C. Chi, Wan L. Huang, Pei W. Hsieh, and Shyng S. Yuan. Cancer Chemotherapy and Pharmacology, June 2017; 79: 1129-1140

References using FLARE™ Assay Technology

Evidence for different mechanisms of action behind the mutagenic effects of 4-NOPD and OPD: the role of DNA damage, oxidative stress and an imbalanced nucleotide pool. Rikard Asgard, Siamak Haghdoost, Siv Osterman Golkar, Bjorn Hellman and Stefan Czene. Mutagenesis, Nov 2013; 28: 637 - 644

Regulation of oxidized base damage repair by chromatin assembly factor 1 subunit A. Chunying Yang, Shiladitya Sengupta, Pavana M. Hegde, Joy Mitra, Shuai Jiang, Brooke Holey, Altaf H. Sarker, Miaw-Sheue Tsai, Muralidhar L. Hegde, and Sankar Mitra. Nucleic Acids Res., Jan 2017; 45: 739 - 748

FOXO3 Transcription Factor Is Essential for Protecting Hematopoietic Stem and Progenitor Cells from Oxidative DNA Damage. Carolina L. Bigarella, Jianfeng Li, Pauline Rimmelé, Raymond Liang, Robert W. Sobol, and Saghi Ghaffari. J. Biol. Chem., Feb 2017; 292: 3005 - 3015

Glucantime® causes oxidative stress-derived DNA damage in Balb/c mice infected by Leishmania (Leishmania) infantum. Vanessa R. Moreira, Luís C. Jesus, Rossy P. Soares, Luis D. Silva, Bruno A. Pinto, Maria N. Melo, Antonio M. de Andrade Paes, and Silma R. Pereira. Antimicrobial Agents and Chemotherapy, June 2017; 61: 02360-16

Trevigen designs, develops and delivers products for Physiologic Cell Culture, Cell Stress and DNA Damage. We specialize in and offer kits and reagents for Organoid Culture, Cancer Cell Behavior, Apoptosis Detection, Oxidative Stress and DNA Damage Detection. Trevigen Cell Assays (TCA) is a branch of Trevigen, conducting contract research work for the pharmaceutical, biotechnology, government and academic segments of the biomedical research market.

R&D SYSTEMS

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BIOLOGICALS

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protein **simple**

A&D

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