



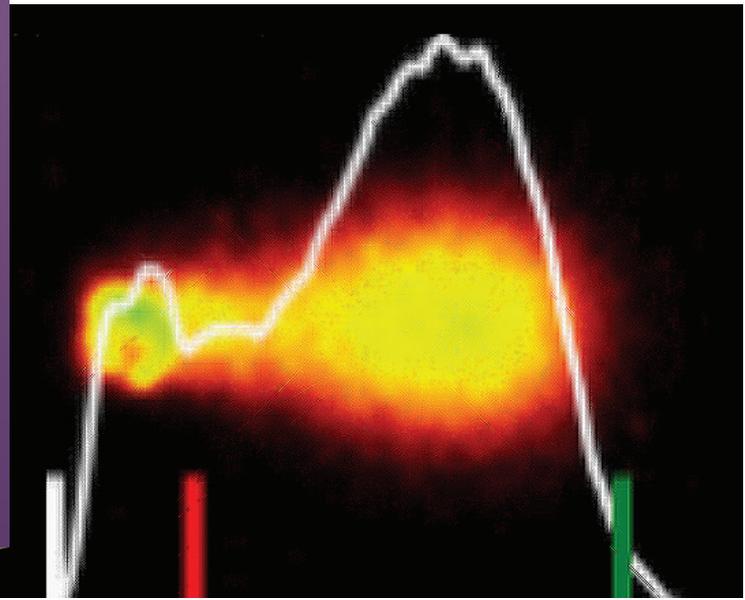
Trevigen®

Better Products. Better Results.

biotechne®

CometAssay® System

*Standardized System
for DNA Damage Analysis*



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.

Software



Instrumentation



Treated Slides



Control Cells



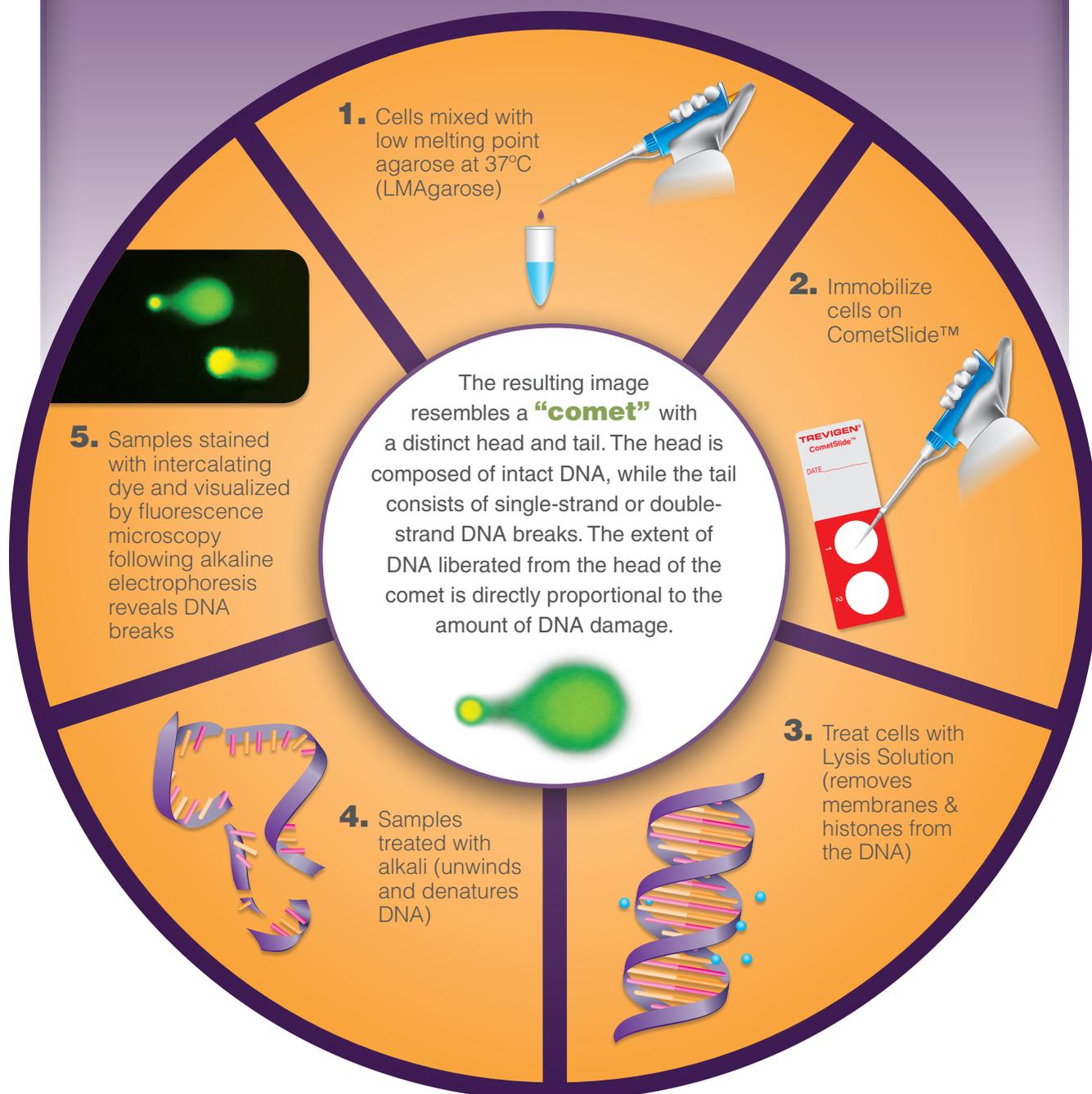
Reagent Kits



Introduction

Method

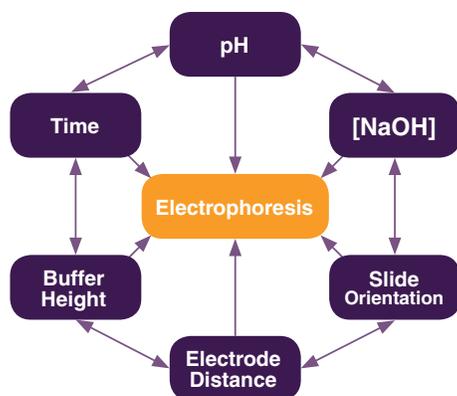
The comet assay is a sensitive technique for quantifying DNA damage and repair from in vivo and in vitro samples of eukaryotic and plant cells. This is the only technique that directly measures DNA damage in individual cells and thus has rapidly gained importance in the fields of genetic toxicology and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



Instrumentation

CometAssay® Electrophoresis System II

Optimized Alkaline
Electrophoresis of CometSlides™



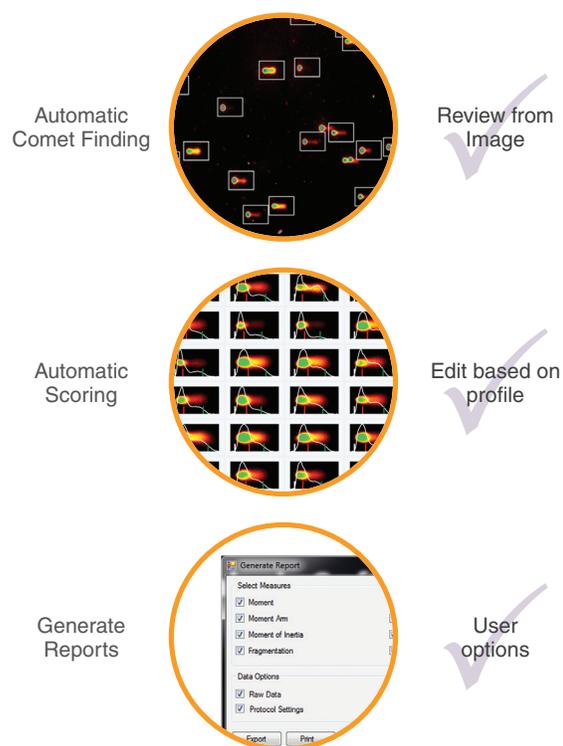
Unique Features

- 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

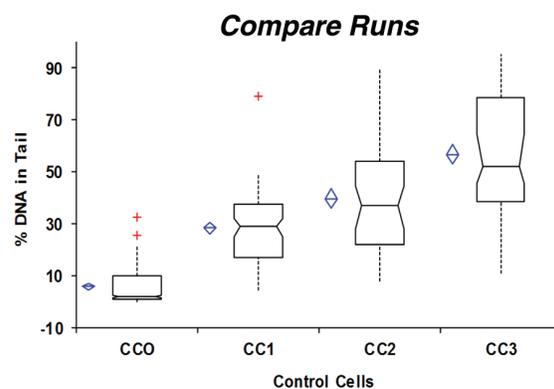
- Use existing microscope and camera for image capture
- Uses 4X and 10X images
- Step by Step Instructions - with Comet Wizard
- Drag and Drop images directly into slide wells
- Automatic Comet finding and Scoring
- Instant and Unbiased Analysis
- Affordable at single flat rate with no service fee

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

Control Cells

CometAssay® Control Cells

Viable cell suspensions with incremental increases in DNA damage



Cryopreserved
Store long term



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 and Neutral Comet Assay
- Cell populations remain stable in liquid nitrogen for long term storage
- Standardized with CometAssay® Electrophoresis System II

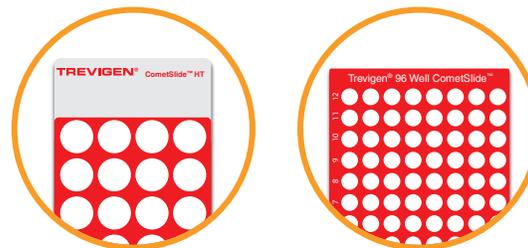
Treated Slides

CometSlides™ and Rack System

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



Multiple Formats
Save Time & Costs



Select Slide Format
Suitable for Your
Research

see Ordering Information



Unique Features

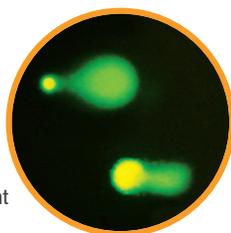
- Specially treated to promote adherence of low melt agarose during alkaline steps
- Hydrophobic barrier for easy application of cells, staining solution, and enzyme treatment to improve assay sensitivity
- The 2 and 3-well slides are interchangeable
- Easily scalable based on slide format to save time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

Reagent Kits

CometAssay® Reagent Kit

Just select slide format to minimize number of runs

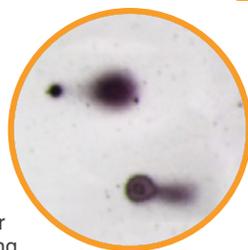
- ✓ Lysis Solution
- ✓ LMAgarose
- ✓ 200 mM EDTA, pH 10
- ✓ CometSlides™
- ✓ Detailed Instructions



Fluorescent Staining

CometAssay® Silver Kit

Allows visualization by standard light microscopy



Silver Staining

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

Reagent Kits with Enzymes

FLARE™ Assay Kits

Fragment Length Analysis using Repair Enzymes in the CometAssay®

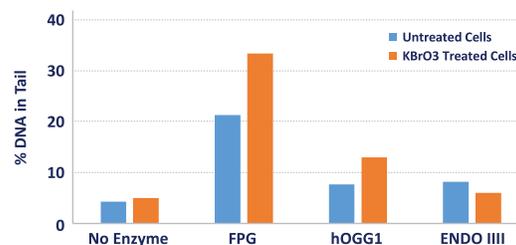
Increase in damage with enzyme addition is indicative of unrepaired but recognized lesions within the DNA.

Step 1: Harvest cells and immobilize in LMAgarose on FLARE™ Slide

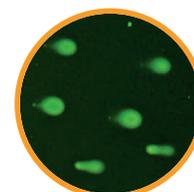
Step 2: After lysis, incubate samples with lesion specific DNA Repair Enzymes

Step 3: Perform alkaline electrophoresis

Comet Assay & DNA Repair Enzymes



No Enzyme - 5 µg/ml MMS



FPG - 5 µg/ml MMS

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



Benefits

- **Qualified Reagents and Enzymes**
increase assay sensitivity
- **Control Cells**
standards to compare runs
- **Treated Slides**
save time and costs with multiple formats
- **Optimized Instrumentation**
minimize electrophoresis variability
- **Analysis Software**
save time and minimize bias
- **Tech Support**
tutorials and webinars
- **Contract Services**
expertise to design studies for your specific needs

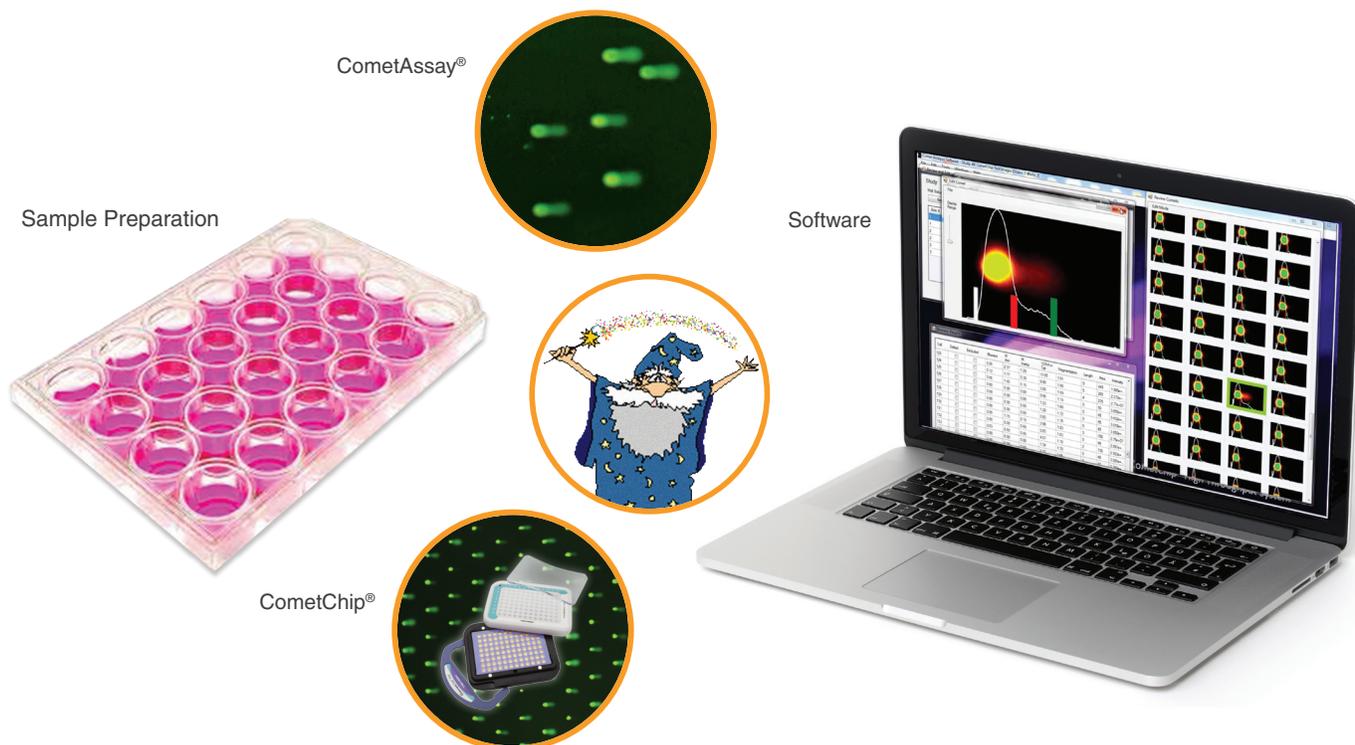
Contract Services

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 cat# 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.



Ordering Information

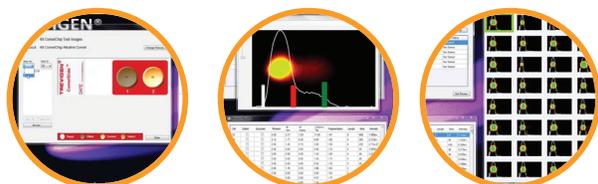
Starter Kits

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).

Catalog #	Component
4250-050-ES	Comet Assay® Electrophoresis System II
4250-050-ESK	CometAssay® 2 Well ES Unit w/ Starter Kit
4250-050-ESK-PS	CometAssay® 2 Well ES Unit w/ Starter Kit and Power Supply
4252-040-ESK	CometAssay® 20 Well ES Unit w/ Starter Kit
4252-040-ESK-PS	CometAssay® 20 Well ES Unit w/ Starter Kit and Power Supply
4253-096-ESK	CometAssay® 96 Well ES Unit w/ Starter Kit
4253-096-ESK-PS	CometAssay® 96 Well ES Unit w/ Starter Kit and Power Supply

Software

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



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

Control Cells

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

Slides and Rack System

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

CometAssay® Reagent Kits

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

Ordering Information

CometAssay® Reagents

Catalog #	Component	Amount
4250-010-01	CometAssay® Lysis Solution	100 ml
4250-050-01	CometAssay® Lysis Solution	2 x 500 ml
4250-050-02	CometAssay® LMAgarose	15 ml
4250-500-02	CometAssay® LMAgarose	100 ml
4250-050-04	200 mM EDTA, pH 10	12.5 ml

FLARE™ Assay Modules and Sample Prep

Catalog #	Component	Amount
3950-075-SP	FLARE™ Sample Prep	75 samples
4040-100-FM	FPG FLARE™ Module	100 samples
4045-01K-FM	E. coli Endonuclease III Module	100 samples
4130-100-FM	hOGG1 FLARE™ Module	100 samples
4867-100	Hydrophobic Coverslips	100 each

FLARE™ Assay Kits

Catalog #	Component	Amount
4040-100-FK	FPG FLARE™ Assay Kit	75 samples
4045-01K-FK	E. coli Endonuclease III FLARE™ Assay Kit	75 samples
4130-100-FK	hOGG1 FLARE™ Assay Kit	75 samples

Contract Services

cat# 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

Related Products

CometChip® Electrophoresis Starter Kit

cat# 4260-096-ESK

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

CometChip® Reagent Kit

cat# 4260-096-K

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

CometChip® Starter Kit

cat# 4260-096-CSK

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

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, PhD
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, PhD
University of New Mexico, Pharmaceutical Sciences

Frequently Asked Questions

1. 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.

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. 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.

4. 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

5. 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.

6. 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.

7. 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.

8. 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.

9. 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.

10. 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.

11. 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.

12. 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.

13. 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.

14. 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.

For more FAQs, please see Helpful Resources at www.trevigen.com

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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.



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8405 Helgerman Ct, Gaithersburg, MD 20877 | 301.216.2800 | www.trevigen.com



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