TREVIGEN® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

CometAssay® ES

Electrophoresis System for the CometAssay®

Catalog # 4250-050-ES

CometAssay® ES

Catalog # 4250-050-ES

Electrophoresis system* for the CometAssay®

Table of Contents

*Patent Pending

I. Introduction

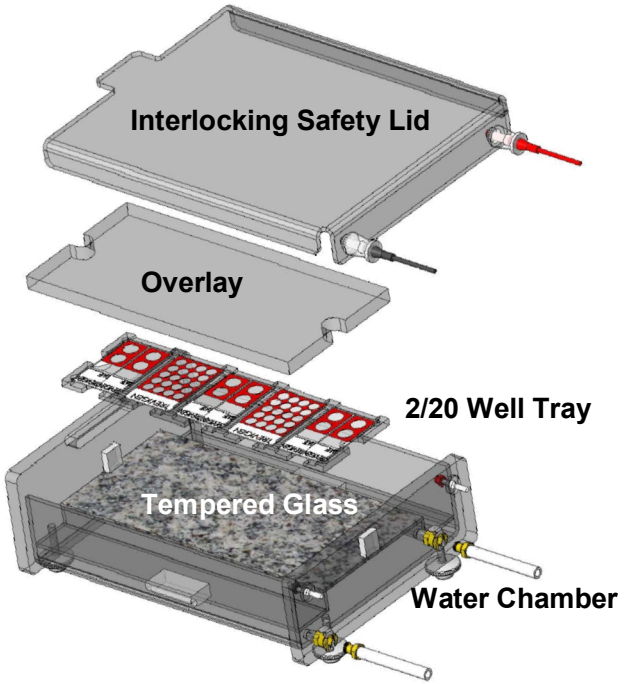
Trevigen's CometAssay® provides a simple and effective method for evaluating DNA damage in cells. The principle of the assay is based upon the ability of denatured, cleaved DNA fragments to migrate out of nucleoids under the influence of an electric field, whereas undamaged DNA migrates slower and remains within the confines of the nucleoid when a current is applied. The sample is then visualized by epi-fluorescence microscopy. Evaluation of the DNA "comet" tail shape and migration pattern allows for assessment of DNA damage.

The Neutral CometAssay® is typically used to detect double-stranded breaks, whereas the Alkaline CometAssay® is more sensitive, and is used to detect smaller amounts of damage including single and double-stranded breaks. In comet assays, cells are immobilized in a bed of low melting point agarose, on a Trevigen CometSlide™. For both assay types, cells are lysed and the nucleoids subjected to electrophoresis. In the Alkaline CometAssay®, samples are treated with alkali to unwind and denature the DNA and hydrolyze sites of damage prior to electrophoresis. Subsequent staining with a fluorescent DNA intercalating dye is performed for visualization. Quantitative and statistical data can readily be generated by analysis of the results using one of several commercially available image analysis software packages which calculate tail length, percent DNA in the tail, and tail moment.

Since variability has been observed when performing the comet assay, Trevigen has developed a CometAssay® Electrophoresis System (ES) (Figure 1) optimized for use with Trevigen's CometAssay® Kits (e.g. cat# 4250-050-K) and Control Cells (cat# 4256-010-CC and cat# 4257-010-NC). Electrophoresis may be performed using either Neutral Electrophoresis Buffer or Alkaline Electrophoresis Solution. Alkaline electrophoresis is more sensitive to variations in buffer height, temperature, and ion concentration, which affect DNA migration and can adversely impact results.

Trevigen's CometAssay® Electrophoresis System overcomes variations first by placing an acrylic overlay on top of an elevated slide tray to maintain optimal buffer height for DNA migration. Secondly, a constant buffer temperature is maintained using an external water chamber to cool the tempered glass slide platform and buffer chamber. Electrophoresis time for optimal DNA migration is achieved in 30 minutes with Alkaline (pH>13) Electrophoresis Solution, or 60 minutes with Neutral Electrophoresis Buffer at 1V/cm. The smoke grey colored unit is designed to minimize exposure to UV light but still allow visual inspection. Third, specially designed slide trays are also provided to accommodate 2, 20 and 96 well slides and maintain proper slide orientation during electrophoresis. Trevigen's CometAssay® Electrophoresis System, alkaline CometAssay® Control Cells (cat# 4256-010-CC), and Neutral CometAssay® Control Cells (cat# 4257-010-NC) enable investigators to consistently optimize their comet assays for highly reproducible results, and to standardize electrophoresis methods between individual users and laboratories.

Figure 1: Trevigen's CometAssay® Electrophoresis System:



II. Safety Information and Warnings

1. **Caution! Electrical Hazard!** This equipment is designed for use with a DC power supply providing up to 250 VDC. Although equipped with a safety interlock system, this apparatus should always be operated with extreme caution. Careless handling can result in electrical shock. To avoid any risk of injury, the instrument should be operated in accordance with the instruction provided. Trevigen is not responsible for any injury or damage caused either by the use of this instrument for purposes other than for which it is intended or by modifications of the instrument not by Trevigen.
2. **Never fill the water chamber without both hoses attached.** Do not exceed a 100 ml/minute fill rate. Attached hoses allow for the release of excess water pressure. Failure to attach can result in the formation of cracks, and can damage the unit.
3. **Do not use ports to recirculate.**
4. **Do not freeze.** Aqueous coolant expansion will damage the unit. Never operate damaged or leaking equipment.
5. The CometAssay® Electrophoresis System is specifically designed with an interlocking safety lid so no part of the active electrophoresis chamber is exposed during operation. Do not attempt to modify this safety design.
6. Always connect the cables to the power supply before turning the power supply on.
7. Never exceed maximum allowed voltage (250V) or current (450 mA).
8. Power to unit is to be supplied by an external DC-voltage power supply.
9. This instrument is designed and certified to meet IEC 1010-1 safety standards.
10. We recommend cleaning the unit with water and nonabrasive soap or detergent, followed by a rinse with deionized water. Avoid abrasive cleaners and rough cloths or brushes. Do not expose the apparatus to window sprays, phenol, acetone, benzene, halogenated hydrocarbon solvents, or undiluted laboratory alcohols.
11. Do not expose the unit to prolonged exposure to UV light, or excessive heat such as dry heat sterilization or autoclaving.

III. Description of Equipment

1. Electrophoresis Tank with Leveling Bubble and Hoses

The electrophoresis system is made of translucent smoke grey acrylic plastic. The deck plate is made of tempered glass, which transfers heat from the slide plate to the surrounding water chamber to help maintain cool and even slide temperature during alkaline electrophoresis. The level of the electrophoresis system is adjusted using the four leveling feet with the leveling bubble resting on top of the deck plate. The enveloping water chamber is filled by attaching **both** hoses to external ports. Care must be taken when filling to remove as much of the air under the deck plate as possible. The recommended buffer volume is 950 ml. Base dimensions are 21 cm x 29 cm.

2. Safety Lid and Cables

The interlocking safety lid is made of black translucent smoke grey acrylic plastic with attached cables. The lid is designed to slide into a rectangular latch. The cables and latch are at opposite ends of the safety lid. Always check that the cables are properly connected to the electrodes.

3. 2/20 (Cat# 4250-050-ES1) and 96 (Cat# 4250-050-ES2) Well Slide Trays

The slide trays, machined from acrylic material, are designed to locate the slides in their running position and for easy slide removal at the completion of electrophoresis. The 2/20 Well Slide Tray holds ten 2-well or five 20-well slides. The 96 Well Slide Tray holds three 96-well slides.

4. Slide Tray Overlay

The 1.3 cm acrylic Overlay is designed to sit on top of the Slide Tray and maintain a constant buffer height (current path) of 0.4 cm above the tray. The volume of electrophoresis buffer in the tank must be filled below the top of the overlay but above its base line for proper operation.

IV. Materials Required But Not Supplied

Equipment:

1. AC Power Supply designed to supply constant voltage, amperage, or power with automatic cross-over. Minimal Output specifications are 21V (constant) with fluctuating amperage to 400 mA.

   Note: Power supplies are included if you order item 4250-050-ESK.

2. 1–20 µl, 20–200 µl, 200–1,000 µl pipettors, and tips
3. Serological pipettor and pipettes
4. Boiling water bath and 37°C water bath
5. Epifluorescence microscope equipped with Fluorescein filter or light transmission microscope when using silver staining kit.
6. 1 L graduated cylinder
7. 4°C refrigerator/cold room

Reagents:

1. Deionized water
2. 10X PBS, Ca^{++} and Mg^{++} free* (cat# 4870-500-6)[1]
3. Ethanol
4. TE Buffer (10 mM Tris (pH 7.5), 1 mM EDTA)
5. CometAssay® Kit (required: e.g. cat# 4250-050-K)[1]

[1] Available from Trevigen; refer to Section XI for ordering information.

For alkaline assays:

6. NaOH Pellets
7. 0.5 M EDTA (pH 8.0)
8. CometAssay® Control Cells (cat# 4256-010-CC)[1]

For neutral assays:

9. 10X Neutral Electrophoresis Buffer
10. Ammonium Acetate
11. Sodium Acetate
12. Glacial Acetic Acid
13. Neutral CometAssay® Control Cells (cat# 4257-010-NC)[1]

Optional reagents:

14. Silver staining kit* (cat# 4254-050-K)
15. Dimethylsulfoxide
16. Tris Borate EDTA buffer

[1] Available from Trevigen; refer to Section XI for ordering information.

V. Reagent Preparation

Prepare one of the following electrophoresis solutions based on the sensitivity of assay desired. Additional reagents are required. For information regarding preparation of all needed reagents, please see the instructions for use for Trevigen's CometAssay® (cat# 4250-050-K).

For Alkaline CometAssay®:

1. Alkaline Electrophoresis Solution pH>13 (200 mM NaOH, 1 mM EDTA)

Prepare a stock solution of 500 mM EDTA, pH 8 (disodium salt)
For 1 liter of electrophoresis solution:

NaOH pellets	8 g
500 mM EDTA, pH 8	2 ml
dH_2O	to 1 liter (after NaOH is dissolved)

Use of freshly made solution is recommended. Prechill at 4°C.

For Neutral CometAssay®:

2. 1X Neutral Electrophoresis Buffer

To prepare 10X **Neutral Electrophoresis Buffer**:

Tris Base (mol. wt. = 121.14)	60.57 g
Sodium Acetate (mol. wt. = 136.08)	204.12 g

Dissolve in 450 ml of dH_2O. Adjust to pH = 9.0 with glacial acetic acid. Adjust volume to 500 ml and filter sterilize. Store at room temperature. Dilute the 10X stock to 1X in dH_2O to prepare 1 liter working strength buffer and prechill at 4°C.

VI. Operation of Equipment

A. Initial Setup

1. Fill water chamber. Stand the unit on end with the hose ports on top. Carefully attach **both** hoses to port plugs on electrophoresis tank by depressing metal clamp on port and inserting each hose. The electrophoresis tank is next placed vertically in a laboratory sink. **Ensure that both valves are open by briefly filling through each hose**. **Never fill the water chamber without the second hose attached to release water pressure or the unit may crack**. **Do not use damaged units.** With one hose connected to a dIH_20 tap and the other positioned to release pressure, fill at no more than 100 ml/min. The water chamber is full when water is released from the second hose. When the hoses are disconnected from the electrophoresis tank the water chamber is sealed. Another option is to fill the tank with cold water if the user is unable to cool electrophoresis unit in cold room or refrigerator.

2. Level electrophoresis tank. Level the electrophoresis tank using the leveling bubble provided by adjusting the four thumbscrew feet.

B. Electrophoresis

1. Cool electrophoresis tank, tray, overlay and lid to 4°C by placing in cold room or refrigerator. Do not freeze the unit. Align the slots of the appropriate slide tray and place on tempered glass platform. Inserting finger slots of slide tray adjacent to cathode (black) electrode is recommended.

2. Prepare Electrophoresis Solution (Buffer). Prepare 1L fresh as described in Reagent Preparation. Cool to 4°C by pouring 950 ml into electrophoresis tank placed in cold room or refrigerator.

3. Perform Alkaline or Neutral Comet Quick Reference (VII).

4. Perform Electrophoresis.

i. Immediately prior to electrophoresis, place electrophoresis unit at room temperature.
ii. Insert slides into tray. Two well slides are locked into position by placing two slides into each position. Recommend always placing slide with Trevigen logo adjacent to cathode (black) electrode. DNA migrates towards the anode (red) during electrophoresis (Figure 1).
iii. The 950 ml of Electrophoresis Solution (Buffer) should completely cover the slides. Carefully insert Slide Tray Overlay over slide tray by aligning slots. To ensure complete buffer coverage of slides, gradually lower Slide Tray Overlay in a manner similar to lowering a slide coverslip.
iv. Verify that the Electrophoresis Solution (Buffer) is not above the Slide Tray Overlay.
v. Set the power supply to 21 V (1 volt per cm). For Alkaline CometAssay® apply voltage for 30 minutes for 2/20 well slides and 40 minutes for 96 well slides. For Neutral CometAssay® apply voltage for 60 minutes for 2/20 well slides.

Alkaline Tips:

The Alkaline Electrophoresis Solution is a non-buffered system and **temperature control is highly recommended.** If the electrophoresis unit and buffer are pre-cooled to 4°C, performance of electrophoresis at room temperature for 30-40 minutes is acceptable. The tempered glass plate maintains the slide temperature.

In-house electrophoresis parameters with 200 mM NaOH/1mM EDTA Alkaline Solution, pH>13 at 4°C, has an amperage of ~220 mA. With increases in buffer temperature, the amperage increase is problematic for some power supplies with higher alkali concentrations. If the user prefers a higher alkali concentration, we recommend running at 0.7 volts per cm with a proportional increase in electrophoresis time.

The Slide Tray Overlay is recommended for all Alkaline Electrophoresis. Any variation in buffer height directly affects DNA migration. Increase in buffer height results in slower DNA migration. The Slide Tray Overlay maintains a buffer height of 0.4 cm.

Neutral Tips:

Use of 1X Neutral Electrophoresis Buffer, based on in house experience, has amperage of ~130 mA when performed at 21V.

The Neutral CometAssay® will detect mainly double-stranded breaks. Neutral CometAssay® images are different from Alkaline CometAssay® images (VIII. Data Analysis, Figures 2 and 3). A high level of damage observed in Alkaline Comet does not necessarily mean damage will be observable using Neutral CometAssay® and longer electrophoresis times (beyond 1 hour) may not necessarily improve results.

VII. Comet Assay Protocols

A. Alkaline Comet Quick Reference

The Assay Protocol described below is written as a Quick Reference using alkaline Comet Control Cells (cat# 4256-010-CC) for two well slides. Reagents and detailed instructions including reagent preparation are provided with Trevigen's CometAssay® Kits (See Section XI).

1. Chill Lysis Solution at 4°C for at least 20 minutes before use.
2. Melt LMAgarose and cool in a 37°C water bath for at least 20 minutes.
3. Combine 50 µl of CCO (control cells) with 500 µl molten LMAgarose (at 37°C) and immediately pipette 50 µl onto two well CometSlide™. Use side of pipette tip to spread agarose/cells over sample area.
4. Repeat step 3 for samples CC1, CC2, and CC3.
5. Place slides at 4°C in the dark for 10 minutes.
6. Immerse slides in prechilled Lysis Solution at 4°C, for 30 minutes.
7. Immerse slides in 50 ml freshly prepared Alkaline Unwinding Solution, pH>13 for 20 minutes at room temperature, in the dark.
8. Perform Alkaline Electrophoresis as described in VI.B.
9. Immerse slides twice in dH_2O for 5 minutes each, then in 70% ethanol for 5 minutes.
10. Dry samples at ≤ 45°C for 10-15 minutes.

11. Place 100 µl of diluted SYBR® Green I[1] onto each sample for 30 minutes. Remove excess SYBR solution. Allow slide to dry completely at room temperature in the dark. Remove excess SYBR solution. Allow slides to dry completely at room temperature in the dark.

12. View slides by epifluorescence microscopy.

B. Neutral Comet Quick Reference

For optimal results, the Neutral CometAssay® should always be performed using Neutral CometAssay® Control Cells (cat# 4257-010-NC). ***Please note that CometAssay® Control Cells (cat# 4256-010-CC) are designed for Alkaline Comet assay only.***

1. Chill Lysis Solution at 4°C for at least 20 minutes before use.
2. Melt LMAgarose and cool in a 37°C water bath for at least 20 minutes.
3. Combine 50 µl of NCO (control cells) with 500 µl molten LMAgarose (at 37°C) and immediately pipette 50 µl onto two well CometSlides™. Use side of pipette tip to spread agarose/cells over sample area.
4. Repeat step 3 for samples NC1, NC2, and NC3, respectively.
5. Place slides at 4°C in the dark for 10 minutes.
6. Immerse slides in prechilled Lysis Solution at 4°C, for 60 minutes.
7. Immerse slides in 50 ml of prechilled 1X Neutral Electrophoresis Buffer for 30 minutes, at 4°C.
8. Perform Neutral Electrophoresis as described in VI.B.
9. Immerse slides in DNA Precipitation Solution for 30 minutes at room temperature.
10. Immerse slides in 70% ethanol for 30 minutes at room temperature.
11. Dry samples at ≤ 45°C for 10-15 minutes.
12. Place 100 µl of diluted SYBR® Green I onto each sample for 30 minutes. Remove excess SYBR solution. Allow slide to dry completely at room temperature in the dark.
13. View slides by epifluorescence microscopy.

[1] SYBR® Green I is a registered product of Molecular Probes, Eugene OR, and is sold under license from Molecular Probes, Inc. Please see p.15 for complete licensing terms. Use of this reagent outside of the scope of these terms is not endorsed by Trevigen,Inc.

VIII. Data Analysis

When excited (425–500 nm) the DNA-bound SYBR® Green I emits green light. In healthy cells the fluorescence is confined to the nucleoid (comprised of high molecular weight DNA): undamaged DNA is supercoiled and thus, does not migrate very far out of the nucleoid under the influence of an electric current. Whereas in cells that have accrued DNA damage, migrating fragments (comet tail) from the nucleoid (comet head) are observed. The negatively charged DNA migrates toward the anode and the extrusion length reflects increasing relaxation of supercoiling, which is indicative of damage. Common descriptors of DNA damage for alkaline comet assays are Percent DNA in the Tail, and Tail Moment. Percent DNA in the Tail is a normalized measure of the percent of total cell DNA

found in the tail. Tail moment is a damage measure combining the amount of DNA in the tail with distance of migration. In neutral comet assays, Tail Moment is primarily used, since tail length continues to increase in contrast to alkaline comet tails which have finite lengths.

Qualitative Analysis (Alkaline CometAssay®)

The comet tail can be scored according to DNA content (intensity). The control (untreated cells) should be used to determine the characteristics of data for a healthy cell. Scoring can then be made according to nominal, medium or high intensity tail DNA content. At least 50 cells should be scored per sample.

Quantitative Analysis (Alkaline and Neutral CometAssay®)

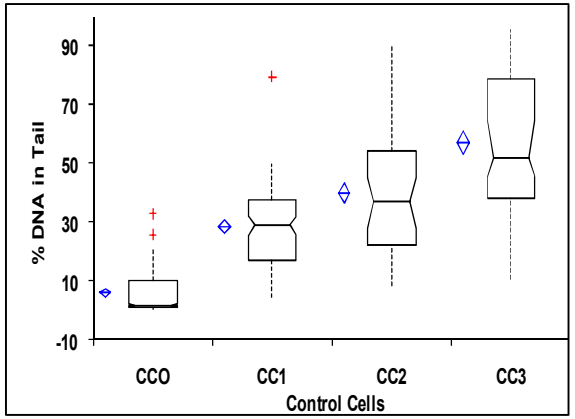
There are several image analysis systems that are suitable for quantitation of CometAssay® data. The more sophisticated systems include the microscope, camera and computer analysis package. These systems can be set up to measure the length of DNA migration, image length, nuclear size, and calculate DNA damage parameters. At least 50 randomly selected cells should be analyzed per sample.

A list of commercially available software package is available from Trevigen.

Alkaline Comet Data

In Figure 2a, data collected for each alkaline CometAssay® Control Cell population (cat# 4256-010-CC) is shown as side-by side vertical box plots for comparison. The diamond shows the mean and confidence interval around the mean. The notched box shows the median, lower and upper quartiles, and the 75% confidence interval around the median. An example is provided below.

Figure 2a: Box-Whisker plot of Control Cells: Percent DNA in Comet Tail



% DNA by Etoposide	n	Mean	SD	SE	75% CI of Mean	Median	IQR	75% CI of Median
CCO	50	5.757	7.7270	1.0928	4.485 to 7.029	1.640	8.925	1.290 to 2.230
CC1	50	28.374	14.0080	1.9810	26.068 to 30.680	28.990	20.313	25.180 to 31.840
CC2	50	39.736	21.8164	3.0853	36.144 to 43.328	37.050	32.183	27.790 to 44.630
CC3	50	56.800	23.5893	3.3360	52.916 to 60.683	51.905	40.240	45.460 to 64.390

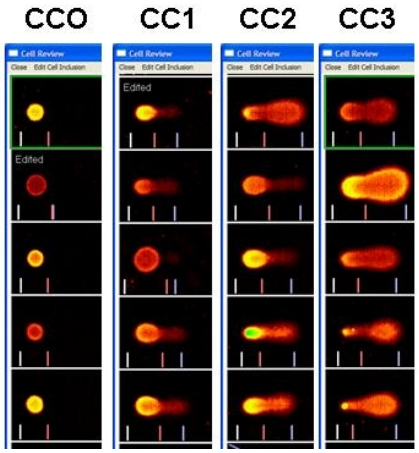
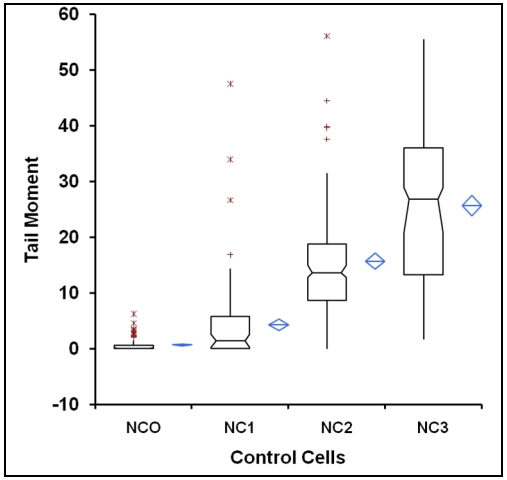


Figure 2b: Example comet tail shapes for each population.

Neutral Comet Data

Data collected for each Neutral CometAssay® Control Cell population (cat# 4257-010-NC) is provided below.

Figure 3a: Box-Whisker plot of Neutral Control Cells: Tail Moment



TM by Bleomycin	n	Mean	SD	SE	75% CI	Median	IQR	75% CI
NCO	75	0.677	1.2410	0.1433	0.511 to 0.843	0.000	0.637	0.000 to 0.140
NC1	75	4.316	7.7817	0.8986	3.274 to 5.358	1.360	5.748	0.240 to 2.510
NC2	75	15.711	10.7829	1.2451	14.268 to 17.155	13.600	10.117	12.830 to 14.950
NC3	75	25.730	13.7918	1.5925	23.884 to 27.577	26.780	22.750	20.810 to 28.930

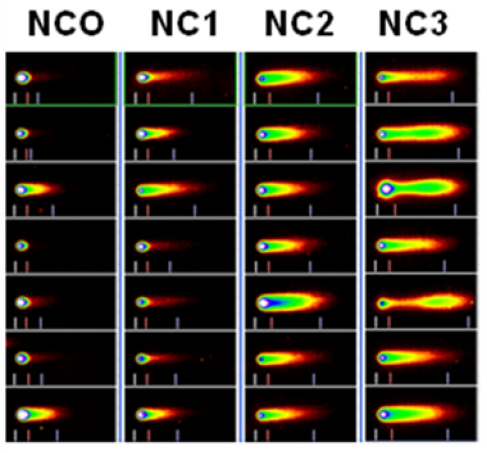


Figure 3b: Example comet tail shapes for each population.

IX. References

1. Lemay, M. and K.A. Wood, 1999. Detection of DNA damage and identification of UV-induced photoproducts using the CometAssay® kit. *BioTechniques* **27**(4):846-851.
2. Angelis, K.J., M. Dusinska and A.R. Collins. 1999. Single cell gel electrophoresis: Detection of DNA damage at different levels of sensitivity. *Electrophoresis* **20:**2133-2138.
3. Morris, E.J., J.C. Dreixler, K-Y. Cheng, P.M. Wilson, R.M. Gin and H.M. Geller. 1999. Optimization of single-cell gel electrophoresis (SCGE) for quantitative analysis of neuronal DNA damage. *BioTechniques* **26:**282-289.
4. Malyapa, R.S., C. Bi, E.W. Ahern, and J.L. Roti, 1998. Detection of DNA damage by the alkali comet assay after exposure to low dose gamma radiation. *Radiation Res* 1**49:**396-400.
5. Henderson, L., A. Wolfreys, J. Fedyk, C. Bourner, S. Windeback, 1998. The ability for the comet assay to discriminate between genotoxins and cytotoxins. *Mutagenesis* **13:**89-94.
6. Visvardis, E.E., A.M. Tassiou, and S.M. Piperakis,1997. Study of DNA damage induction and repair capacity of fresh cryopreserved lymphocytes exposed to H_2O_2 and γ-irradiation with the alkaline comet assay. *Mutation Res* **383:**71-80.
7. Fairbairn, D.W., P.L. Olive, K.L. O'Neill. 1995. The comet assay: a comprehensive review. *Mutation Res* **339:**37-59.
8. Collins, A.R., A.G. Ma, and S.J. Duthie, 1995. The kinetics of repair of oxidative DNA damage (strand breaks and oxidized pyrimidine dimers) in human cells. *Mutation Res* **336:**69-77.
9. Singh, N.P., M.T. McCoy, R.R. Tice, and E.L. Schneider, 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res* **175:**184 -191.
10. Östling, O. and K. J. Johanson, 1984. Microelectrophoretic study of radiation- induced DNA damage in individual cells. *Biochem Biophys Res Commun* **123:**291-298.
11. Singh, N.P., R.E. Stephens, 1997. Microgel electrophoresis: sensitivity, mechanisms, and DNA electrostretching. *Mutation Res* **383:**167–175.

X. Troubleshooting Guide

General Problems

PROBLEM	CAUSE	ACTION
Unexpected and/or variety of tail shape.	LMAgarose too hot	Cool LMAgarose to 37°C before adding cells.
Cells in LMAgarose did not remain attached to the CometSlide™.	Electrophoresis solution too hot.	Control temperature performing electrophoresis at 4°C.
	Cells were not washed to remove medium before combining with LMAgarose.	The pH of medium and carry over serum proteins, etc., can reduce the adherence of the agarose. Resuspended cells in 1X PBS.
	Agarose percentage was too low.	Do not increase ratio of cells to molten agarose by more than 1 to 10.
	LMAgarose was not fully set before samples were processed.	Ensure 0.5 mm dried ring due to agarose disc retraction is seen at the edge of the CometSlide™ area.
	LMAgarose unevenly set on the slide.	Spread the agarose with the side of a pipette tip to ensure uniformity of agarose disc and better adherence.
	Rinsing steps too harsh.	Gently place slides into solutions. Do not pour solutions over slides.

Specific to Alkaline Comet

PROBLEM	CAUSE	ACTION
Majority of cells in untreated control sample have large comet tails.	Unwanted damage to cells occurred in culture or in sample preparations	Check morphology of cells to ensure healthy appearance.
		Handle cells or tissues gently to avoid physical damage.
	Electrophoresis solution too hot	Control temperature by performing electrophoresis at 4°C.
	Intracellular activity	Keep cells on ice and prepare cell samples immediately before combining with molten LMAgarose.
Majority of cells in untreated control sample have small to medium comet tails.	Endogenous oxidative damage or endonuclease activity after sample preparation is damaging DNA.	Ensure Lysis solution was chilled before use.
		Add DMSO to any cell sample that may contain heme groups.
		Ensure PBS used is calcium and magnesium free.
		Work under dimmed light conditions or under yellow light.
In positive control (*e.g.* 100 µM hydrogen peroxide for 30 minutes on ice) no evidence of comet tail.	No damage to DNA.	Use fresh hydrogen peroxide to induce damage.
	Sample was not processed correctly.	Ensure each step in protocol was performed correctly. Failure to lyse, denature in alkali, or to properly perform electrophoresis may generate poor results.

PROBLEM	CAUSE	ACTION
Comet tails present but not significant in positive control.	Insufficient denaturation in Alkaline Solution.	Increase time in Alkaline Solution up to 1 hour.
	Insufficient electrophoresis time.	Increase time of electrophoresis up to up to 1 hour for alkaline electrophoresis. Increase time of electrophoresis when running at cold temperatures.

Specific to Neutral Comet

PROBLEM	CAUSE	ACTION
In positive control no evidence of comet tail.	Damaging agent doesn't cause double-strand breaks.	Confirm damage by Alkaline Comet.
		Run Neutral Control Cells to confirm electrophoresis conditions.
		Increase treatment with damaging agent.
In positive control comet tails are extremely long and do not fit analysis window.	Cells are necrotic or apoptotic.	Verify 75% viability. Decrease treatment with damaging agent.
	Electrophoresis time too long.	Decrease electrophoresis time to 15-30 minutes.
Diffusion Artifacts are present; Difficulty distinguishing between head and tail	Electrophoresis buffer conditions may not be optimal	Use 1X Neutral Electrophoresis Buffer and follow protocol on page 7.

XI. Related Products Available From Trevigen

Contact Trevigen for details of our unique product line for studying DNA damage and repair. All of Trevigen's kits include highly qualified enzymes, substrates, buffers, full instructions for use, and a synopsis specific for your kit.

CometAssay® Kits:

Catalog #	Description	Size
4250-050-ESK	CometAssay® Starter Kit	each
4250-040-K	CometAssay®	40 samples
4251-050-K	CometAssay® Silver Kit	50 samples
4254-200-K	CometAssay® Silver Staining Kit	200 samples
4252-040-K	CometAssay® Higher Throughput Kit	40 samples
4253-096-K	CometAssay® Kit 96 Wells	96 samples

Control Cells:

Catalog #	Description	Size
4256-010-CC	CometAssay® Control Cells (alkaline assay)	1 set
4257-010-NC	Neutral CometAssay® Control Cells	1 set

FLARE™ Assay Kits:

Catalog #	Description	Damage Recognized	Size
4040-100-FK	Fpg Kit	8-oxoguanine, DNA containing formamidopyrimidine moieties	75 samples
4040-100-FM			100 samples

FLARE™ Assay Kits (cont.):

Catalog #	Description	Damage Recognized	Size
4055-100-FK 4055-100-FM	T4-PDG Kit	Cis-syn isomers of cyclo-butane pyrimidine dimers	75 samples 100 samples
4065-100-FK 4065-100-FM	cv-PDG Kit	Cis-syn and trans-syn isomers of cyclobutane pyrimidine dimers	75 samples 100 samples
4100-100-FK 4100-100-FM	UVDE Kit	Cyclobutane pyrimidine dimers, (6-4) photoproducts	75 samples 100 samples
4130-100-FK 4130-100-FM	hOGG1 Kit	8-oxoguanine, DNA containing formamidopyrimidine moieties	75 samples 100 samples
4045-01K-FK 4045-01K-FM	Endonuclease III Kit	Thymine Glycol, 5,6-dihydro-thymine, urea, 5-hydroxy-6-hydrothy-mine, 5,6-dihydro-uracil, alloxan, 5-hydroxy-6-hydrouracil, uracil glycol, 5-hydroxy-5-methylhy-dantoin, 5-hydroxycytosine,5-hydroxy-uracil, methyl-tartonylurea, thymine ring saturated or fragmentation product	75 samples 100 samples

PARP Assay Kits:

Catalog #	Description	Size
4520-096-K	HT PARP in vivo Pharmacodynamic Assay II	96 tests
4684-096-K	HT Colorimetric PARP/Apoptosis Assay	96 samples
4676-096-K	Universal Chemiluminescent PARP Assay w/Histone Coated Strip Wells	96 samples

Accessories:

Catalog #	Description	Size
4250-050-03	CometSlide™ (2 well)	25 slides
4252-200-01	CometAssay® HT Slide (20 well)	10 slides
4253-960-03	96 Well CometSlide™	10 slides
3950-300-02	FLARE™ Slides	100 slides
4867-100	Hydrophobic Coverslips	100 each
4040-100-FM	Fpg FLARE™ Module	>100 samples
4130-100-FM	hOGG1 FLARE™ Module	>100 samples
4045-100-FM	Endonuclease III FLARE™ Module	>100 samples
4055-100-FM	T4-PDG FLARE™ Module	>100 samples
4065-100-FM	cv-PDG FLARE™ Module	>100 samples
4100-050-FM	UVDE FLARE™ Module	>100 samples
3950-075-SP	FLARE™ Sample Prep	>100 samples
4667-250-01	Recombinant Human PARP Enzyme	250 µl
4668-100-1	Recombinant Human PARP (High Specific Activity)	1000 Units
4354-MC-050	Anti-8-oxo-dG (clone 2E2)	50 µl
4370-096-K	HT 8-oxo-dG ELISA Kit	96 wells

XII. Appendices

Appendix A

Neutral CometAssay®

The CometAssay® may be performed using neutral conditions that employ 1X TBE. Without treatment with Alkaline Buffer, this Neutral CometAssay® will also detect mainly double-stranded breaks.

1. Chill Lysis Solution at 4°C for at least 20 minutes before use.
2. Melt LMAgarose and cool in a 37°C water bath for at least 20 minutes.
3. Combine 50 µl of cells at 1 x 10^5/ml with 500 µl molten LMAgarose (at 37°C) and immediately pipette 50 µl onto two well CometSlides™. Use side of pipette tip to spread agarose/cells over sample area.
4. Repeat step 3 for remaining samples.
5. Place slides at 4°C in the dark for 10 minutes.
5. Immerse slides in prechilled Lysis Solution at 4°C for 30 minutes.
6. Immerse slides in 50 ml of 4°C 1X TBE buffer for 15 minutes.

To prepare 10X TBE:

Tris Base	108 g
Boric Acid	55 g
EDTA (disodium salt)	9.3 g

Dissolve in 900 ml dH_2O. Adjust volume to 1 liter and filter sterilize. Store at room temperature. Dilute the 10X TBE to 1X in dH_2O to prepare 1 liter working strength buffer and store at 4°C.

7. For the CometAssay® ES tank, add 4°C 950 ml 1X TBE Buffer, place slides in electrophoresis slide tray and cover with Slide Tray Overlay. Set power supply to 21 volts and apply voltage for 40 minutes.
8. Immerse slides in dH_2O for 5 minutes.
9. Immerse slides in 70% ethanol for 5 minutes.
10. Dry samples at ≤ 45°C for 10-15 minutes.
11. Place 100 µl of diluted SYBR® Green I onto each sample and place in refrigerator for 5 minutes. Remove excess SYBR solution. Allow slide to dry completely at room temperature in the dark.
12. View slides by epifluorescence microscopy.

Appendix B

Alternative Alkaline Electrophoresis Solution

300 mM NaOH, 1 mM EDTA pH>13:

For 1 liter of electrophoresis solution:

NaOH pellets	12 g
500 mM EDTA, pH 8	2 ml
dH_2O (after NaOH is dissolved) add to:	1 liter

Use of freshly made solution is recommended. Prechill at 4°C.

This amperage is increased to ~350 mM with 300 mM NaOH/1mM EDTA at 4°C and electrophoresis increased to 40 minutes for 2 and 20 well slides.

SYBR® Green I nucleic acid gel stain licensing terms:

This product is sold under license from Molecular Probes, Inc. under US Patents Nos. 5,436,134 and 5,658,751 for use in a comet assay for internal research and development only, where research and development use expressly excludes the use of this product for providing medical, diagnostic or any other testing analysis or screening services or providing clinical information or clinical analysis, in return for compensation on a per-test basis, and research and development use expressly excludes incorporation of this product into another product for commercialization even if such other product would be commercialized for research and/or development use.

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes.

Trevigen, Inc.
8405 Helgerman Ct. Gaithersburg, MD 20877
Tel: 1-800-873-8443 • 301-216-2800
Fax: 301-560-4973
e-mail: info@trevigen.com
www.trevigen.com

Please Recycle