Product Manual

Comet SCGE assay kit

Catalog # ADI-900-166

Reagent kit for Single Cell Electrophoresis Assay
50 tests (2 tests/slide)

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Product Manual

Reagents require separate storage conditions.

Check our website for additional protocols, technical notes and FAQs.

For proper performance, use the insert provided with each individual kit received.

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INTRODUCTION

The Comet SCGE Assay is a single cell gel electrophoresis assay that 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 the cell 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. Evaluation of the DNA “comet” tail shape and migration pattern allows for assessment of DNA damage. In this assay, cells are immobilized in a bed of low melting point agarose on a Comet Slide. Following a gentle cell lysis, samples are treated with alkali to unwind and denature the DNA and hydrolyze sites of damage. The samples are then submitted to electrophoresis and staining with a fluorescent DNA intercalating dye. The sample is then visualized by epifluorescence microscopy. As an alternative for researchers who do not have access to a fluorescence microscope, silver staining allows standard light microscopy for data analysis.

The electrophoresis step may be performed using TBE buffer or alkaline electrophoresis solution. TBE circumvents the problems associated with use of non-buffered alkali for electrophoresis, such as poor DNA migration due to saturation of charge by sodium ions, and difficulties in controlling voltage. TBE is preferred for analysis of apoptosis and enables use of the tail length, rather than the tail moment, for data analysis. The alkaline electrophoresis is more sensitive and will detect smaller amounts of damage. The electrophoresis time may be extended up to 40 minutes when running at very low amperage. Data may be analyzed qualitatively if the comets are scored according to categories of small to large tail lengths. 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 and tail moment.

The Comet SCGE Assay’s Comet Slide is specially treated to promote adherence of low melting point agarose. This eliminates the time consuming and unreliable traditional method of preparing base layers of agarose. The use of Comet Slides shortens assay time, and allows the rapid and reliable analysis of large numbers of samples.

MATERIALS SUPPLIED

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

The materials should be handled with care due to the known and unknown effects of the antigen.

1. Lysis Solution

   2 x 500 mL, Catalog No. 4250-050-01

   2.5M NaCl, 100 mM EDTA (pH10), 10 mM Tris Base, 1% sodium lauryl sarcosinate, 1% Triton x-100

2. Comet LMAgarose

   15 mL, Catalog No. 4250-050-02

   Low melting point agarose in PBS

3. Comet Slide

   25 each, Catalog No. 4250-050-03

4. EDTA (200 mM)

   12.5 mL, Catalog No. 4250-050-04

5. CYGREEN® Nucleic Acid Dye

   20 µL, Catalog No. ENZ-GEN105-0020

STORAGE

Store CYGREEN® Nucleic Acid Dye at -20°C. All other reagents are stable at room temperature.

OTHER MATERIALS NEEDED

1. 1-20 µL, 20-200 µL, 200-1000 µL pipettors, and tips
2. Serological pipettor and pipets
3. Boiling water bath and 37˚C water bath
4. Horizontal electrophoresis apparatus
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
8. Peristaltic pump for recirculation of buffer (optional).
9. 10X PBS, Ca^{2+} and Mg^{2+} free
10. NaOH Pellets
11. Dimethylsulfoxide (DMSO) (optional)
12. 10X TBE Buffer (required for neutral electrophoresis)
13. 0.5 M EDTA (pH8.0) (required for alkaline electrophoresis)
14. Ethanol
15. Silver staining kit (optional)
16. TE Buffer (10 mM Tris (pH 7.5), 1 mM EDTA)
17. Deionized water

REAGENT PREPARATION

Reagents marked with an asterisk (*) should be prepared immediately before use.

1. **1X PBS, Ca^{2+} and Mg^{2+} free**

   Dilute 10X PBS with deionized water to prepare 1X PBS. Store at room temperature.

2. **Lysis Solution**

   For up to 10 slides (2 samples per slide) prepare:

Lysis Solution	40 mL
DMSO	4 mL (optional)

   Chill at 4°C, or on ice, for at least 20 minutes before use. The addition of DMSO is optional and is required only for samples containing heme, such as blood cells or tissue samples.

3. **Comet LMAgarose**

   The Comet LMAgarose is ready to use once molten. Loosen the cap to allow for expansion then heat the bottle in a 90-100˚C water bath for 5 minutes, or until the agarose is molten (Caution: Microwaving is not recommended). Place the bottle in a 37°C water bath for at least 20 minutes to cool. The LMAgarose will remain molten at 37°C for sample preparation indefinitely. The LMAgarose formulation is proprietary.

4. **Alkaline Solution, pH>13***

   Wear gloves when preparing and handling the Alkaline Solution. Per 50 mL of Alkaline Solution combine:

NaOH Pellets	0.6 g
200 mM EDTA	250 µL
Deionized Water	49.75 mL

   Stir until fully dissolved. The solution will warm during preparation. Allow to cool to room temperature before use.

5. **Electrophoresis Solution**

   Prepare one of the following electrophoresis solutions based on the sensitivity of assay desired.

   a. 1X TBE Electrophoresis Buffer

   To prepare 10X TBE:

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

   Dissolve in 900 mL of deionized water. Adjust volume to 1 liter and autoclave. Store at room temperature. Dilute the 10X TBE to 1X in deionized water to prepare working strength buffer.

   Or

   b. Alkaline Electrophoresis Solution pH >13 (300 mM NaOH, 1 mM EDTA)

   Prepare a stock solution of 500 mM EDTA, pH8. For 1 L of electrophoresis solution:

NaOH pellet	12 g
500 mM EDTA, pH 8	2 mL

   Deionized Water to 1 liter (after NaOH is dissolved) Adjust the volume prepared based on the dimensions of your electrophoresis apparatus. Use of freshly made solution is recommended.

6. **Staining Solution**

   Prepare Staining Solution from the CYGREEN® Nucleic Acid Dye provided (10,000x concentrate in DMSO).

CYGREEN® Dye	1 µL
Deionized Water	999 µL

   Note: The final concentration is 10x.

   The diluted stock is stable for several weeks when stored at -20˚C in the dark.

7. **Anti-fade Solution (Not needed for CYGREEN® Dye. For Silver Staining.)**

   Prepare if fading of samples occurs. In a 50 mL tube, mix until dissolved:

p-Phenylenediamine dihydrochloride	500 mg
1X PBS	4.5 mL

Add approximately 400 µL of 10 N NaOH dropwise with stirring until pH of solution reaches 7.5-8.0. Add 1X PBS to increase the volume to 5 mL, and 45 mL of glycerol for a final volume of 50 mL. Vortex mixture thoroughly and apply

10 µL per sample, covering samples with coverslip. Nail polish may be used to seal coverslip. Re-staining of slides is not recommended. Store anti-fade solution at -20°C for up to one month. Darkening of solution may occur.

8. **Comet Slide**

   Do not wash slides; they are washed during the manufacturing process. Spots that may appear on the slides are normal. Washing slides may result in removal of the proprietary coating solution. We assume no responsibility for the performance of the slides if they are washed.

SAMPLE HANDLING

Cell samples should be prepared immediately before starting the assay, although success has been obtained using cryopreserved cells (see below). Cell samples should be handled under dimmed or yellow light to prevent DNA damage from ultraviolet light. Buffers should be chilled to 4˚C or on ice to inhibit endogenous damage occurring during sample preparation and to inhibit repair in the unfixed cells. PBS must be calcium and magnesium free to inhibit endonuclease activities. The appropriate controls should also be included (see below). Optimal results in the Comet SCGE Assay are usually obtained with 500-1000 cells per Comet Slide sample area. Using 50 µL of a cell suspension at 1 x 10^5 cells per mL combined with 500 µL of LMAgarose will provide the correct agarose concentration and cell density for optimal results when plating 75 µL per sample.

Suspension Cells

Cell suspensions are harvested by centrifugation. Resuspend cells at 1 x 10^5 cells/mL in ice cold 1X PBS (Ca^{2+} and Mg^{2+} free). The media used for cell culture can reduce the adhesion of the agarose on the Comet Slide.

Adherent Cells

Gently scrape cells using a rubber policeman. Transfer cells and medium to centrifuge tube, perform cell count, then pellet cells. Wash once in ice cold 1X PBS (Ca^{2+} and Mg^{2+} free). Resuspend cells at 1 x 10^5 cells/mL in ice cold 1X PBS (Ca^{2+} and Mg^{2+} free).

Tissue Preparation

Place a small piece of tissue into 1-2 mL of ice cold 1X PBS (Ca^{2+} and Mg^{2+} free),

20 mM EDTA. Using small dissecting scissors, mince the tissue into very small pieces and let stand for 5 minutes. Recover the cell suspension, avoiding transfer of debris. Count cells, pellet by centrifugation, and resuspend at 1 x 10^5 cells/mL in ice cold 1X PBS (Ca^{2+} and Mg^{2+} free). For blood rich organs (e.g., liver, spleen), chop tissue into large pieces (1-2 mm^3), let settle for 5 minutes then aspirate and discard medium. Add 1-2 mL of ice cold 20 mM EDTA in 1X PBS (Ca^{2+} and Mg^{2+} free), mince the tissue into very small pieces and let stand for 5 minutes. Recover the cell suspension, avoiding transfer of debris. Count cells, pellet, and resuspend at 1 x 10^5 cells/mL in ice cold 1X PBS (Ca^{2+} and Mg^{2+} free).

Controls

A sample of untreated cells should always be processed to control for endogenous levels of damage within cells, and for damage that may occur during sample preparation. Control cells and treated cells should be handled in an identical manner. If UV damage is being studied, the

cells should be kept in low level yellow light during processing. If you require a sample that will be positive for comet tails, treat cells with 100 µM hydrogen peroxide or 25 µM $KMnO_4$ for 20 minutes at 4˚C.

Treatment will generate significant oxidative damage in the majority of cells, thereby providing a positive control for each step in the comet assay. Note that the dimensions and characteristics of the comet tail, as a consequence of H_2O_2 or $KMnO_4$ treatment, may be different to those induced by the damage under investigation.

Method for Cryopreservation of Cells Prior to use in the Comet SCGE Assay

Certain cells, e.g., lymphocytes, may be successfully cryopreserved prior to performing the Comet SCGE Assay (Visvardis, ***et al***.). A pilot study should be performed to determine if cryopreservation is appropriate for the cells in use.

1. Centrifuge cells at 200 x g for 5 minutes.
2. Resuspend cell pellet at 1 x 10^7 cells/mL in 10% (v/v) dimethyl-sulfoxide, 40% (v/v) medium, 50% (v/v) fetal calf serum.
3. Transfer aliquots of 2 x 10^6 cells into freezing vials.
4. Freeze at -70˚C with -1˚C per minute freezing rate.
5. Recover cells by submerging in 37˚C water bath until the last trace of ice has melted.
6. Transfer to 15 mL of prechilled 40% (v/v) medium, 10% (w/v) dextrose, 50% (v/v) fetal calf serum.
7. Centrifuge at 200 x g for 10 minutes at 4˚C.
8. Resuspend in ice cold 1X PBS (Ca^{2+} and Mg^{2+} free) and proceed with the Comet SCGE Assay.

ASSAY PROCEDURE

Both protocols provided are for alkali unwinding conditions. The electrophoresis conditions will determine the sensitivity of the assay. TBE electrophoresis or Neutral electrophoresis after alkali unwinding will detect single-stranded DNA breaks, double-stranded DNA breaks, and may detect a few apurinic sites, apyrimidinic sites. Alkaline electrophoresis will detect single-stranded DNA breaks, double-stranded DNA breaks, and the majority of apurinic sites, apyrimidinic sites as well as alkali labile DNA adducts (e.g. phosphoglycols, phosphotriesters). The Comet SCGE Assay has been reported to detect DNA damage associated with low doses (0.6 cGy) of gamma irradiation, providing a simple technique for quantitation of low levels of DNA damage. Prior to performing the Comet SCGE Assay, a viability assay should be performed to determine the dose of the test substance that gives at least 75% viability. False positives may occur when high doses of cytotoxic agents are used. For information on performing the neutral comet assay that will predominantly detect double-stranded DNA breaks, see Appendix A. For cryopreservation of cells, fixing the Comet Slide samples, and storage, refer to Sample Handling.

The Comet SCGE Assay requires approximately 2-3 hours to complete, including the incubations and electrophoresis. Once the cells or tissues have been prepared, the procedure is not labor intensive. The Lysis Solution may be chilled and the LMAgarose melted while the cell and tissue samples are being prepared.

All steps are performed at room temperature unless otherwise specified. Work under dimmed or yellow light to prevent damage from UV.

1. Prepare Lysis Solution and chill at 4˚C or on ice for at least 20 minutes before use.
2. Melt LMAgarose in a beaker of boiling water for 5 minutes, with the cap loosened. Place bottle in a 37˚C water bath for at least 20 minutes to cool. The temperature of the agarose is critical or the cells may undergo heat shock. Heat blocks are not recommended for regulating the temperature of the agarose.

3. Combine cells at 1 x 10^5/ mL with molten LMAgarose (at 37˚C) at a ratio of 1:10 (v/v) and immediately pipette 75 µL onto the Comet Slide. If necessary, use side of pipette tip to spread agarose/cells over sample area to ensure complete coverage of the sample area. When working with many samples it may be convenient to place aliquots of the molten agarose into prewarmed microcentrifuge tubes and place the tubes at 37˚C. Add cells to one tube, mix by gently pipetting once or twice, then transfer 75 µL aliquots onto each sample area as required. Then proceed with the next sample of cells.

   Comet LMAgarose (molten and at 37˚C from step 2) 500 µL

   Cells in 1X PBS (Ca^{2+} and Mg^{2+} free) at 1 x 10^5/ mL 50 µL

   Note: If sample is not spreading evenly on the slide, warm the slide at 37˚C before application.

4. Place slide flat at 4˚C in the dark (e.g. place in refrigerator) for 10 minutes. A 0.5 mm clear ring appears at edge of the Comet Slide area. Increasing gelling time to 30 minutes improves adherence of samples in high humidity environments.
5. Immerse slide in prechilled Lysis Solution and leave on ice, or at 4˚C, for 30 to 60 minutes.
6. Tap off excess buffer from slide and immerse in freshly prepared Alkaline Solution, pH>13 (see Reagent Preparation). WEAR GLOVES WHEN PREPARING OR HANDLING THIS SOLUTION.
7. Leave the Comet Slide in Alkaline Solution for 20 to 60 minutes at room temperature, in the dark.

   To perform TBE Electrophoresis go to step 8 or for Alkaline go to step 13.

8. Remove slide from Alkaline Solution, gently tap excess buffer from slide and wash by immersing in 1X TBE buffer for 5 minutes, 2 times (see Reagent Preparation).
9. Transfer slide from 1X TBE buffer to a horizontal electrophoresis apparatus. Place slides flat onto a gel tray and align equidistant from the electrodes. Pour 1X TBE buffer until level just covers samples. Set power supply to 1 volt per cm (measured electrode to electrode). Apply voltage for 10 minutes.
10. Very gently tap off excess TBE, and dip slide in 70% ethanol for 5 minutes.
11. Air dry samples. Drying brings all the cells in a single plane to facilitate observation. At this stage, samples may be stored at room temperature, with desiccant.

    NOTE: Silver staining allows visualization of comets on any transmission light microscope and permanently stains the samples for archiving and long term storage. It is recommended that samples

be dried before silver staining.

12. Proceed to Step 16.

For Alkaline Electrophoresis

13. Transfer slide from Alkaline Solution to a horizontal electrophoresis apparatus. Place slides flat onto a gel tray and align equidistant from the electrodes. Carefully pour the Alkaline Solution until level just covers samples. Set the voltage to about 1 volt/cm. Add or remove buffer until the current is approximately 300 mA and perform electrophoresis for 20-40 minutes.

    Tips:

    Since the Alkaline Electrophoresis Solution is a non-buffered system, temperature control is highly recommended. In-house testing has shown great temperature fluctuations when conducting the alkaline electrophoresis at ambient temperature. To improve temperature control, the use of a large electrophoresis apparatus (25-30 cm between electrodes) is recommended along with recirculation of the electrophoresis solution. Alternatively, performing the electrophoresis at cooler temperatures (e.g. 16˚C or 4˚C) will diminish background damage, increase sample adherence at high pHs and significantly improves reproducibility. Choose the method that is most convenient for your laboratory and always use the same conditions, power supplies and electrophoresis chambers for comparative analysis.

14. Gently tap off excess electrophoresis solution, rinse by dipping several times in deionized water, then immerse slide in 70% ethanol for 5 minutes.

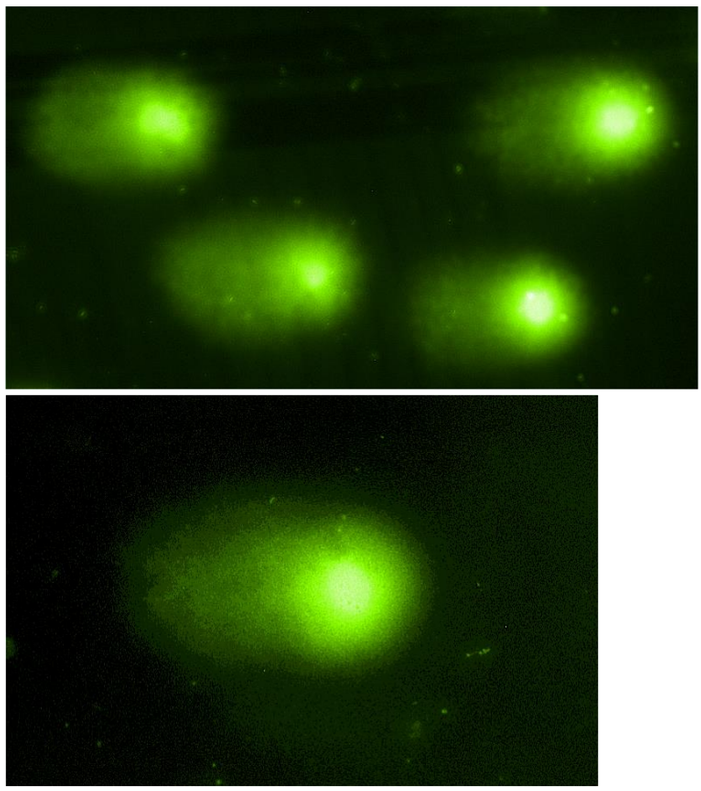
15. Air dry samples. Drying brings all the cells in a single plane to facilitate observation. Samples may be stored at room temperature, with desiccant prior to scoring at this stage.

    NOTE: Silver staining allows visualization of comets on any transmission light microscope and permanently stains the samples for archiving and long term storage. It is recommended that samples be dried before silver staining.

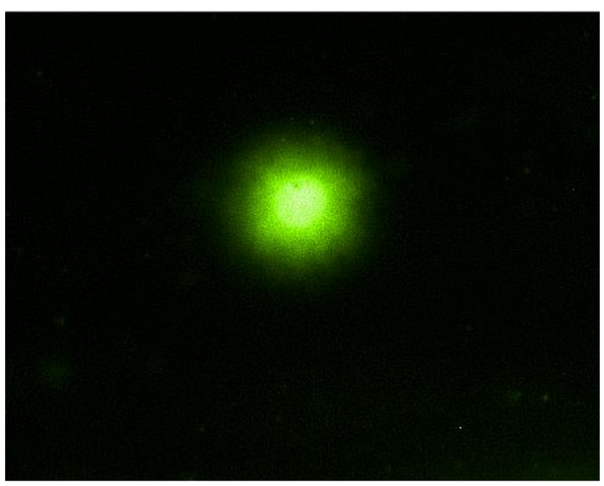
16. Place 100 µL of diluted stain (see Reagent Preparation section) onto each circle of dried agarose and stain for 30 minutes (room temperature) in the dark. Gently tap slide to remove excess stain solution and rinse briefly in water. Allow slides to dry completely at 37°C.

17. View slide by epifluorescence microscopy. FITC filter is recommended for the CYGREEN® Dye (excitation/emission 489/515 nm)

TYPICAL RESULTS



Positive control: CaSki cells were treated with 100 mM Hydrogen peroxide for 20 min at 4°C. Comet SCGE Assay was performed and slides were imaged using a FITC filter.



Negative control: CaSki cells were untreated. Comet SCGE Assay was performed and slides were imaged using a FITC filter

DATA ANALYSIS

When excited, CYGREEN® Nucleic Acid Dye emits green light. In healthy cells the fluorescence is confined to the nucleoid: undamaged DNA is supercoiled and thus does not migrate very far of the nucleoid under the influence of an electric current. In cells that have accrued damage to the DNA, the alkali treatment unwinds the DNA, releasing fragments that migrate from the cell when subjected to an electric field. The negatively charged DNA migrates toward the anode and the extrusion length reflects increasing relaxation of supercoiling, which is indicative of damage. When using TBE as the electrophoresis buffer, the length of the comet tail may be correlated with DNA damage. When using alkaline electrophoresis conditions, the distribution of DNA between the tail and the head of the comet should be used to evaluate the degree of DNA damage. The characteristics of the comet tail including length, width, and DNA content may also be useful in assessing qualitative differences in the type of DNA damage.

Qualitative Analysis

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 75 cells should be scored per sample.

Quantitative Analysis

There are several image analysis systems that are suitable for quantitation of Comet SCGE Assay data. The more sophisticated systems include the microscope, camera and computer analysis package. These systems can be set up to establish the length of DNA migration, image length, nuclear size, and calculate the tail moment. At least 75 randomly selected cells should be analyzed per sample.

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APPENDIX A: NEUTRAL COMET SCGE ASSAY

The Comet SCGE Assay may be performed using neutral conditions. Without treatment with Alkaline Buffer, the Neutral Comet SCGE Assay will detect mainly double-stranded breaks and can be useful for assessing the DNA fragmentation associated with apoptosis.

1. Prepare Lysis Solution (see Reagent Preparation) and chill at 4˚C or on ice for at least 20 minutes before use.
2. Melt LMAgarose in a beaker of boiling water for 5 minutes, with the cap loosened, and then cool in a 37˚C water bath for at least 20 minutes.
3. Combine cells at 1 x 10^5/mL with molten LMAgarose (at 37˚C) at a ratio of 1:10 (v/v) and immediately pipette 75 µL onto a Comet Slide. Use side of pipette tip to spread agarose/cells over sample area.

   Comet LMAgarose (molten and at 37˚C from step 2) 500 µL

   Cells in 1X PBS (Ca^{2+} and Mg^{2+} free) at 1 x 10^5/ mL 50 µL

   Note: If sample is not spreading evenly on the slide, warm the slide at 37 ˚C before application.
4. Place slides flat at 4˚C in the dark (e.g. place in refrigerator) for 10 minutes. A 0.5 mm clear ring appears at edge of the Comet Slide area. Increasing gelling time to 30 minutes improves adherence of samples in high humidity environments.
5. Immerse slide in prechilled (Step 1) Lysis Solution and leave on ice or at 4˚C for 30 minutes.
6. Remove slide from Lysis Buffer, tap excess buffer from slide and wash slide by immersing in 50 mL of 1X TBE buffer (see Reagent Preparation).
7. Transfer slides from 1X TBE buffer, and place flat onto a gel tray submerged in 1X TBE buffer in a horizontal electrophoresis apparatus. Align slides equidistant from the electrodes. Set power supply to 1 volt/cm (measured electrode to electrode). Apply voltage for 10-20 minutes.
8. Tap off excess TBE, rinse slides briefly in deionized water.
9. Immerse slide in 70% ethanol for 5 minutes.

10. Air dry samples. Drying brings all the cells in a single plane to facilitate observation. Samples may be stored at room temperature, with desiccant prior to scoring at this stage.

    NOTE: Silver staining allows visualization of comets on any transmission light microscope and permanently stains the samples for archiving and long term storage. It is recommended that samples be dried before silver staining.

11. Place 100 µL of diluted stain on to each sample of dried agarose and stain 30 minutes (room temperature) in the dark. Gently tap slide to remove excess CYGREEN® Dye solution and rinse briefly in water. Allow slides to dry completely at 37°C.

12. View slide by epifluorescence microscopy. FITC filter is recommended for the CYGREEN® Dye (excitation/emission 489/515 nm)

APPENDIX B: INSTRUCTIONS FOR ALKALINE COMET ASSAY WITH OTHER ELECTROPHORESIS UNITS

Since the Alkaline Electrophoresis Solution is a non-buffered system, **temperature control is highly recommended.** In-house testing has shown great temperature fluctuations when conducting the alkaline electrophoresis at ambient temperature. To improve temperature control, the use of a large electrophoresis apparatus (20-30 cm between electrodes) is recommended. Performing the electrophoresis at a cooler temperature (*e.g.* 4°C) will diminish background damage, increase sample adherence at high pHs and significantly improve reproducibility. Choose the method that is most convenient for your laboratory and always use the same conditions, CometAssay® Control Cells (cat# 4256-010-CC), power supplies and electrophoresis chambers for comparative analysis.

ALTERNATIVE REAGENTS

1. Alkaline Unwinding Solution, pH>13 (300 mM NaOH, 1 mM EDTA)

Wear gloves when preparing and handling the Alkaline Unwinding Solution.

Per 50 mL of Alkaline Solution combine:

NaOH Pellets	0.6 g
200 mM EDTA (cat # 4250-050-04)	250 µL
dH2O	49.75 mL

Stir until fully dissolved. The solution will warm during preparation. Allow to cool to room temperature before use.

2. Alkaline Electrophoresis Solution pH >13 (300 mM NaOH, 1 mM EDTA) for other electrophoresis systems:

Prepare a stock solution of 500 mM EDTA, pH 8.

For 1 liter of electrophoresis solution:

NaOH pellets	12 g
500 mM EDTA, pH 8	2 mL
dH2O (after NaOH is dissolved) add to:	1 liter

Adjust the volume prepared based on the dimensions of your electrophoresis
apparatus. Use of freshly made solution is recommended. Cool to 4°C.
Align slides equidistant from electrodes and carefully add 300 mM NaOH (1 mM
EDTA) Alkaline Solution until level just covers samples. Set the voltage to about

1 Volt/cm. Add or remove buffer until the current is approximately 300 mA and
perform electrophoresis for 20–40 minutes.

APPENDIX C: DNA STAINS

1. Important parameters to consider in choosing a DNA stain for the alkaline comet assay are similar fluorescence and decay rates for single- and double strand DNA.

Table 1: DNA Stains Parameters (Cosa *et al*.)

Dye Abs/Em (nm)	**ss:dsDNA fluorescence**	**ss:dsDNA decay**	**Signal:Bkgrd**
EtBr 520/608	1.0	0.89	~10
DAPI 356/455	0.55	0.85	~20
Propidium Iodide 536/624	0.93	0.93	~20
SYBR Gold 496/540	0.84	0.74	>1000
SYBR Green 496/522	0.57	0.47	>1000
YoYo-1 490/507	0.66	0.73	~400

2. To use SYBR Green instead of SYBR Gold, simply prepare 1:10,000X
SYBR® Green I Staining Solution. The diluted stock is stable for several weeks
when stored at 4°C in the dark.
SYBR® Green I (10,000X concentrate in DMSO) 1 µL
TE Buffer, pH 7.5 10 mL
(TE: 10 mM Tris-HCl pH 7.5, 1 mM EDTA)

TROUBLESHOOTING GUIDE

Problem	Cause	Action
Majority of cells in un-treated control sample have large comet tails.	Unwanted damage to cells occurred in culture or in sample preparations. • Electrophoresis solution too hot. • Intracellular activity.	• Check morphology of cells to ensure healthy appearance. • Handle cells or tissues gently to avoid physical damage. • Control temperature by recirculating the electrophoresis solution or performing the assay at less than 20°C. • Keep cells on ice and prepare cell samples immediately before combining with molten LMAgarose.
Unexpected and/or variety of tail shape	LMAgarose too hot.	Cool LMAgarose to 37°C before adding cells. Do not use heat block.
Majority of cells in un-treated 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. 100µM hydrogen peroxide for 30 minutes on ice) no evidence of comet tail.	• No damage to DNA. • Sample was not processed correctly.	• Use fresh hydrogen peroxide to induce damage. • Ensure each step in protocol was performed correctly. Failure to lyse, denature in alkali (optional), or to properly perform electrophoresis may generate poor results.
Comet tails present but not significant in positive control.	• Insufficient denaturation in Alkaline Solution. • Insufficient electrophoresis time.	• Increase time in Alkaline Solution up to 1 hour. • Increase time of electrophoresis up to 20 minutes for TBE and up to 1 hour for alkaline electrophoresis. • Increase time of electrophoresis when running at cold temperature.
Cells in LMAgarose did not remain attached to the CometSlide™.	• Electrophoresis solution too hot. Cells were not washed to remove medium before combining with LMAgarose. • Agarose percentage was too low. • LMAgarose was not fully set before samples were processed. • LMAgarose unevenly set on the slide.	• Control temperature by recirculating the electrophoresis solution or performing the assay at less than 20°C. • The pH of medium and carry over serum proteins, etc., can reduce the adherence of the agarose. Resuspend cells in 1X PBS. • Do not increase ratio of cells to molten agarose by more than 1 to 10. Ensure 0.5 mm dried ring due to agarose disc retraction is seen at the edge of the CometSlide area. • Spread the agarose with the side of a pipette tip to ensure uniformity of agarose disc and better adherence.

NOTES

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