NOTE: Revision to Assay Protocol

Product Manual

OxiSelect™ Comet Assay Kit (3-Well Slides)

Catalog Number

STA-351	75 assays
STA-351-5	5 x 75 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

DNA damage, due to environmental factors and normal metabolic processes inside the cell, occurs at a rate of 1,000 to 1,000,000 molecular lesions per cell per day. While this counts for only a small part of the human genome's approximately 6 billion bases (3 billion base pairs), unrepaired lesions to critical genes can impede a cell's ability to carry out its function and appreciably increase the likelihood of cancer.

The comet assay, or single cell gel electrophoresis assay (SCGE), is a common technique for measurement of DNA damage in individual cells. Under an electrophoretic field, damaged cellular DNA (containing fragments and strand breaks) is separated from intact DNA, yielding a classic "comet tail" shape under the microscope. Extent of DNA damage is usually visually estimated by comet tail measurement; however, image analysis software is also available for measuring various parameters.

The OxiSelect™ Comet Assay is a fast and sensitive kit for the measurement of cellular DNA damage. Each kit provides sufficient reagents to perform up to 75 assays.

Assay Principle

The Cell Biolabs OxiSelect™ Comet Assay is a single cell gel electrophoresis assay (SCGE) for simple evaluation of cellular DNA damage. First, individual cells are mixed with molten agarose before application to the OxiSelect™ Comet Slide. These embedded cells are then treated with a lysis buffer and alkaline solution, which relaxes and denatures the DNA. Finally, the samples are electrophoresed in a horizontal chamber to separate intact DNA from damaged fragments. Following electrophoresis, the samples are dried, stained with a DNA dye, and visualized by epifluorescence microscopy. Under these conditions, the damaged DNA (containing cleavage and strand breaks) will migrate further than intact DNA and produce a "comet tail" shape (see Figure 1). Each kit provides sufficient reagents to perform up to 75 assays.

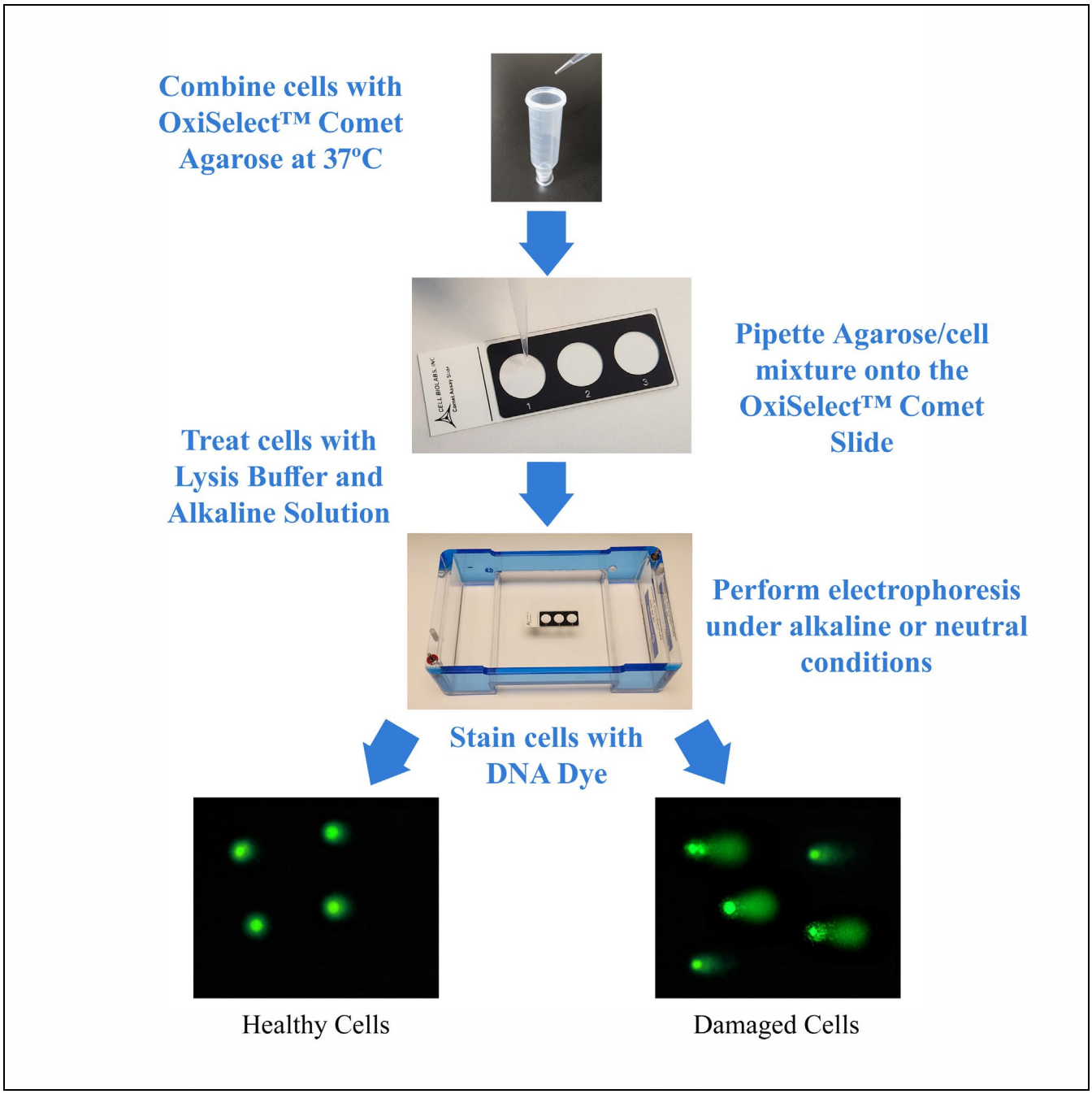

Figure 1: Comet Assay Principle

Related Products

1. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
2. STA-321: OxiSelect™ DNA Double-Strand Break (DSB) Staining Kit
3. STA-324: OxiSelect™ Oxidative DNA Damage Quantitation Kit (AP sites)
4. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
5. STA-350: OxiSelect™ Comet Assay Kit (3-Well Slides), 15 Assays

Kit Components (shipped at room temperature)

1. OxiSelect™ 3-Well Comet Slides (Part No. STA-353): 25 slides.
2. OxiSelect™ Comet Agarose (Part No. 235002): One sterile 15 mL bottle.
3. Vista Green DNA Dye, 10000X (Part No. 235003): One 5 µL vial.
4. EDTA Solution, 500 mM (Part No. 235102): One 250 mL bottle.
5. 10X Lysis Solution (Part No. 235103): One 100 mL bottle.

Materials Not Supplied

1. NaCl powder
2. NaOH pellets
3. 10 N NaOH for pH adjustment
4. DMSO (optional)
5. 70% Ethanol
6. TE Buffer (10 mM Tris, pH 7.5, 1 mM EDTA)
7. PBS (without Mg^{2+} and Ca^{2+})
8. EDTA (disodium salt)
9. DI H_2O

Storage

Upon receipt, store the Vista Green DNA Dye at -20ºC. Store all other kit components at room temperature.

Preparation of Reagents

- OxiSelect™ Comet Agarose: Heat the Comet Agarose bottle at 90-95ºC in a water bath for 20 minutes, or until agarose liquefies. Transfer the bottle to a 37ºC water bath for 20 minutes and maintain until needed.
- Vista Green DNA Dye: Prepare a 1X Vista Green DNA Staining Solution by diluting the provided stock 1:10000 in TE Buffer (10 mM Tris, pH 7.5, 1 mM EDTA). The solution can be stored at 4ºC for up to 3 weeks, protected from light.
- Lysis Buffer: To prepare 100 mL of 1X Lysis Buffer

NaCl	14.6 g
EDTA Solution (provided)	20.0 mL
10X Lysis Solution (provided)	10.0 mL
DMSO	10.0 mL (optional for heme containing samples)
DI H_2O	Adjust volume to 90 mL

Mix thoroughly to dissolve NaCl. Slowly adjust the Lysis Buffer to pH 10.0 with 10 N NaOH, then QS to 100 mL with DI H_2O. Chill Lysis Buffer to 4 ºC before use.

Note: Buffer will appear cloudy at room temperature, but will clear at 4ºC. pH will also remain ~10.0.

- Alkaline Solution: To prepare 100 mL of Alkaline Solution

NaOH	1.2 g
EDTA Solution (provided)	0.2 mL
DI H_2O	Adjust volume to 100 mL

Mix thoroughly to dissolve NaOH. Chill Alkaline Solution to 4ºC before use.

- Electrophoresis Running Solution: Choose the appropriate electrophoresis solution based on the desired running conditions and assay sensitivity. TBE is preferred for analysis of apoptosis and enables use of the tail length, rather than the tail moment, for data analysis. TBE electrophoresis will detect single-stranded and double-stranded DNA breaks, and may detect a few AP sites. Alkaline electrophoresis is more sensitive and will detect smaller amounts of DNA damage. Alkaline electrophoresis will detect single-stranded and double-stranded DNA breaks, the majority of AP sites, and alkali labile DNA adducts.

  To prepare 1 L of Electrophoresis Solution

  1. **TBE Electrophoresis Solution**

Tris Base	10.8 g
Boric Acid	5.5 g
EDTA (disodium salt)	0.93 g
DI H_2O	Adjust volume to 1 L

Mix thoroughly to dissolve solids. Chill TBE Running Solution to 4ºC before use.

OR 2. Alkaline Electrophoresis Solution (300 mM NaOH, pH >13, 1 mM EDTA)

NaOH	12.0 g
EDTA Solution (provided)	2.0 mL
DI H_2O	Adjust volume to 1 L

Mix thoroughly to dissolve NaOH. Chill Alkaline Running Solution to 4ºC before use.

Special Precautions

To avoid ultraviolet light damage to cell samples, perform the assay under low/dim light conditions.

Preparation of Samples and Slides

1. Prepare Lysis Buffer, Alkaline Solution, and Electrophoresis Running Solution (see Preparation of Reagents) prior to performing the assay. Chill all solutions to 4ºC thoroughly.
2. Heat OxiSelect™ Comet Agarose to 90-95ºC in a water bath for 20 minutes, or until agarose liquefies. Cool the agarose by transferring the bottle to a 37ºC water bath for 20 minutes.
3. Prepare cell samples, including controls, as follows:
   - Suspension Cells: Centrifuge cells at 700 x g for 2 minutes and discard supernatant. Wash cell pellet once with ice-cold PBS (without Mg^{2+} and Ca^{2+}), centrifuge, and discard the supernatant. Finally, resuspend the cells at 1 x 10^5 cells/mL in ice-cold PBS (without Mg^{2+} and Ca^{2+}).
   - Adherent Cells: Gently remove cells from flask/dish by scraping with a rubber policeman. Transfer cell suspension to a conical tube and centrifuge at 700 x g for 2 minutes, discarding the supernatant. Wash cell pellet once with ice-cold PBS (without Mg^{2+} and Ca^{2+}), centrifuge, and discard the supernatant. Finally, resuspend the cells at 1 x 10^5 cells/mL in ice-cold PBS (without Mg^{2+} and Ca^{2+}).
   - Tissue Preparation: Using dissection scissors, mince a small piece of tissue in 1-2 mL of ice-cold PBS containing 20 mM EDTA (without Mg^{2+} and Ca^{2+}). Allow the tissue/cell suspension to stand for 5 minutes before transferring the supernatant to a centrifuge tube; avoid transferring debris. Centrifuge, discarding the supernatant, and then resuspend the cells at 1 x 10^5 cells/mL in ice-cold PBS (without Mg^{2+} and Ca^{2+}).
4. Combine cell samples with Comet Agarose (step 2) at 1:10 ratio (v/v), mix well by pipetting, and immediately transfer 75 µL/well onto OxiSelect™ Comet Slide. **Ensure complete well coverage by spreading the solution over the well with the pipette tip.**

   Note: For multiple samples, warm the slide at 37ºC, maintain suspensions at 37ºC to avoid gelation. Titrate samples again just prior to slide addition.
5. Maintaining the slide horizontally, transfer the slide to 4ºC in the dark for 15-30 minutes.
6. Carefully, transfer the slide to a small basin/container containing pre-chilled Lysis Buffer (~25 mL/slide). Immerse the slide in the buffer for 30-60 minutes at 4ºC in the dark.
7. Carefully, aspirate the Lysis Buffer from the container and replace with pre-chilled Alkaline Solution (~25 mL/slide). Immerse the slide in the solution for 30 minutes at 4ºC in the dark.

Assay Protocol

I. TBE Electrophoresis

1. Aspirate the Alkaline Solution from the container and replace with pre-chilled TBE Electrophoresis Solution. Immerse the slide for 5 minutes, and then repeat once more.
2. Maintaining the slide horizontally, carefully transfer the slide to a horizontal electrophoresis chamber. Fill the chamber with cold TBE Electrophoresis Solution until the buffer level covers the slide.
3. Apply voltage to the chamber for 10-15 minutes at 1 volt/cm (e.g., if the chamber electrodes are 35 cm apart, you would then apply 35 volts to the slide)

4. Maintaining the slide horizontally, carefully transfer the slide from the electrophoresis chamber to a clean, small basin/container containing pre-chilled DI H_2O (~25 mL/slide). Immerse the slide for 2 minutes, aspirate, and then repeat twice more.
5. Aspirate the final water rinse and replace with cold 70% Ethanol for 5 minutes.
6. Maintaining the slide horizontally, remove the slide from the 70% Ethanol and allow slide to dry at 37ºC for 30 min.
7. Once the agarose and slide are completely dry, add 100 µL/well of diluted Vista Green DNA Dye (see Preparation of Reagents). Incubate at room temperature for 15 minutes.
8. View slides by epifluorescence microscopy using a FITC filter.

II. Alkaline Electrophoresis

1. Maintaining the slide horizontally, carefully transfer the slide from the Alkaline Solution to a horizontal electrophoresis chamber. Fill the chamber with cold Alkaline Electrophoresis Solution until the buffer level covers the slide.
2. Apply voltage to the chamber for 15-30 minutes at 1 volt/cm (e.g., if the chamber electrodes are 35 cm apart, you would then apply 35 volts to the slide). Additionally, adjust the volume of Alkaline Electrophoresis Solution to produce a current setting of 300 mA.
3. Maintaining the slide horizontally, carefully transfer the slide from the electrophoresis chamber to a clean, small basin/container containing pre-chilled DI H_2O (~25 mL/slide). Immerse the slide for 2 minutes, aspirate, and then repeat twice more.
4. Aspirate the final water rinse and replace with cold 70% Ethanol for 5 minutes.
5. Maintaining the slide horizontally, remove the slide from the 70% Ethanol and allow slide to dry at 37ºC for 30 min.
6. Once the agarose and slide are completely dry, add 100 µL/well of diluted Vista Green DNA Dye (see Preparation of Reagents). Incubate at room temperature for 15 minutes.
7. View slides by epifluorescence microscopy using a FITC filter.

Example of Results

The following figures demonstrate typical OxiSelect™ Comet Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

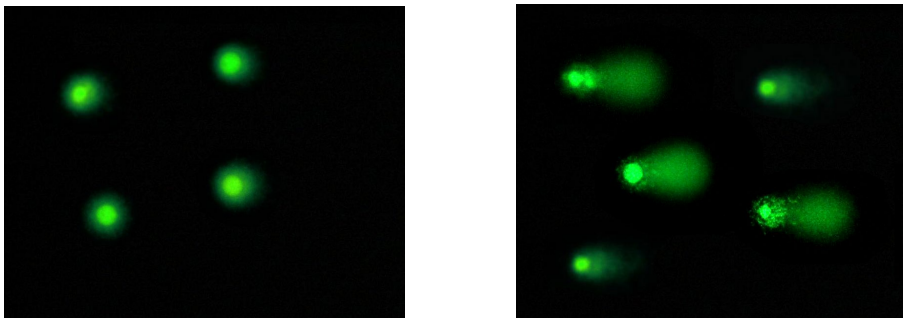

Figure 2. Etoposide Treatment of Jurkat Cells. Jurkat cells were untreated (left) or treated (right) with 20 µM Etoposide for 4 hours before performing Comet Assay (alkaline electrophoresis conditions, 33 V/300 mA for 15 minutes).

Calculation of Results

The DNA damage is quantified by measuring the displacement between the genetic material of the nucleus ('comet head') and the resulting 'tail'. Tail Moment and Tail DNA% are the two most common parameters to analyze Comet assay results. At least 50 -100 cells should be analyzed per sample. The Tail Moment has been suggested to be an appropriate index of induced DNA damage in considering both the migration of the genetic material as well as the relative amount of DNA in the tail.

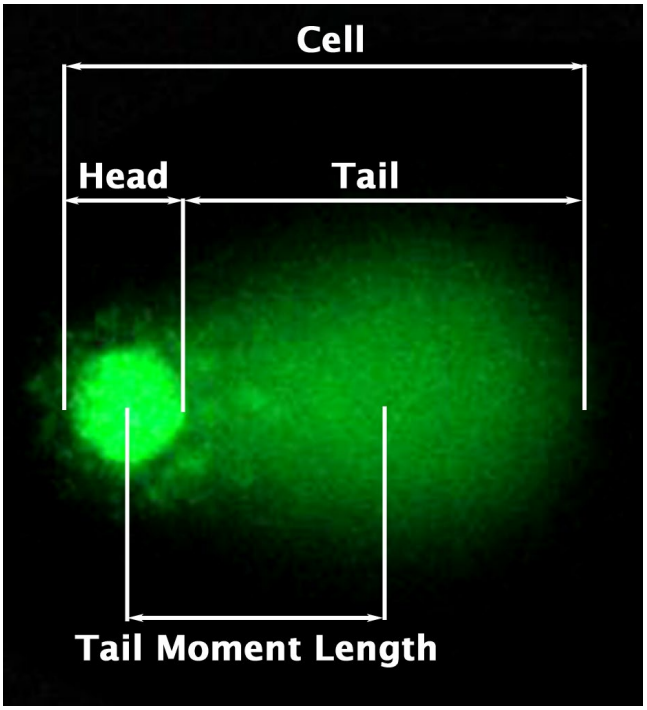

Figure 3: Typical Damaged DNA in Comet Assay.

Tail DNA% = 100 x Tail DNA Intensity/Cell DNA Intensity

Tail Moment can be measured using one of the following methods:

(a) Olive Tail Moment = Tail DNA% x Tail Moment Length*

(b) Extent Tail Moment = Tail DNA% x Length of Tail (see Figure 3)

A number of Comet Assay analysis software programs are commercially available, such as and Comet Assay IV (Perceptive Instruments) and CASPlab.

*Tail Moment Length is measured from the center of the head to the center of the tail (see Figure 3)

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