

Datasheet for ABIN2345016

OxiSelect™ Comet Assay Kit (3-Well Slides)

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Overview

Quantity:	75 tests
Reactivity:	Others
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	The Cell Biolabs OxiSelect™ Comet Assay is a single cell gel electrophoresis assay (SCGE) for simple evaluation of cellular DNA damage.
Brand:	OxiSelect™
Characteristics:	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.
Components:	<ol style="list-style-type: none">OxiSelect™ 3-Well Comet Slides : 25 slides.OxiSelect™ Comet Agarose : One sterile 15 mL bottle.Vista Green DNA Dye, 10000X : One 5 µL vial.EDTA Solution, 500 mM : One 250 mL bottle.10X Lysis Solution : One 100 mL bottle.
Material not included:	<ol style="list-style-type: none">NaCl powderNaOH pellets10 N NaOH for pH adjustmentDMSO (optional)70 % EthanolTE Buffer (10 mM Tris, pH 7.5, 1 mM EDTA)PBS (without Mg²⁺ and Ca²⁺)EDTA (disodium salt)DI H₂O

Product Details

10. 37 °C and boiling water baths
11. Horizontal electrophoresis chamber
12. Adjustable single channel micropipettes with disposable tips
13. Epifluorescence microscope with FITC filter 4

Target Details

Background: 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.

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Application Notes: Optimal working dilution should be determined by the investigator.

Comment:

- Useful screening tool for various types of DNA damage
- Slides are specially treated for adhesion of low-melting agarose
- Easy visualization by epifluorescence microscopy

Protocol: 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 . Each kit provides sufficient reagents to perform up to 75 assays.

Reagent Preparation:

- OxiSelect™ Comet Agarose: Heat the Comet Agarose bottle at 90-95 °C in a water bath for 20 minutes, or until agarose liquefies. Transfer the bottle to a 37 °C 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 4 °C 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 H₂O 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 H₂O. Chill Lysis Buffer to 4 °C before use. Note: Buffer will appear cloudy at room temperature, but will clear at 4 °C. 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 H₂O Adjust volume to 100 mL Mix thoroughly to dissolve NaOH. Chill Alkaline Solution to 4 °C 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 5 Boric Acid 5.5 g EDTA (disodium salt) 0.93 g DI H₂O Adjust volume to 1 L Mix thoroughly to dissolve solids. Chill TBE Running Solution to 4 °C 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 H₂O Adjust volume to 1 L Mix thoroughly to dissolve NaOH. Chill Alkaline Running Solution to 4 °C 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 4 °C thoroughly. 2. Heat OxiSelect™ Comet Agarose to 90-95 °C in a water bath for 20 minutes, or until agarose liquefies. Cool the agarose by transferring the bottle to a 37 °C 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 Mg²⁺ and Ca²⁺), centrifuge, and discard the supernatant. Finally, resuspend the cells at 1 x 10⁵ cells/mL in ice-cold PBS (without Mg²⁺ and Ca²⁺).
 - 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 Mg²⁺ and Ca²⁺), centrifuge, and discard the supernatant. Finally, resuspend the cells at 1 x 10⁵ cells/mL in ice-cold PBS (without Mg²⁺ and Ca²⁺).
 - Tissue Preparation: Using dissection scissors, mince a small piece of tissue in 1-2 mL of ice cold PBS containing 20 mM EDTA (without Mg²⁺ and Ca²⁺). 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 10⁵ cells/mL in ice-cold PBS (without Mg²⁺ and Ca²⁺). 4. Combine cell samples

with Comet Agarose (step 2) at 1:10 ratio (v/v), titrate to mix, and immediately pipette 75 μ L/well onto the OxiSelect™ Comet Slide. Ensure complete well coverage by spreading the suspension over the well with the pipette tip. Note: For multiple samples, maintain suspensions at 37 °C to avoid gelation. Titrate samples again just prior to slide addition. 5. Maintaining the slide horizontally, transfer the slide to 4 °C in the dark for 15 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 4 °C 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 4 °C in the dark.

Assay Procedure:

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 H₂O (~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 to air dry.
7. Once the agarose and slide is completely dry, add 100 μ L/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 H₂O (~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 to air dry.
6. Once the agarose and slide is completely dry, add 100 μ L/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. 7

Calculation of Results:

The DNA damage is quantified by measuring the displacement between the genetic material of

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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. 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) Figure 3: Typical Damaged DNA in Comet Assay. 8

Restrictions: For Research Use only

Handling

Storage: RT/-20 °C

Storage Comment: Upon receipt, store the Vista Green DNA Dye at -20°C. Store all other kit components at room temperature.

Publications

Product cited in: Tse, Chen, Fu, Su, Li, Guo, Zhu, Kwan, Cheng, Cao, Lee, Fong, Yu: "Sensitization of melanoma cells to alkylating agent-induced DNA damage and cell death via orchestrating oxidative stress and IKK β inhibition." in: **Redox biology**, Vol. 11, pp. 562-576, (2017) ([PubMed](#)).

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