Product Data Sheet

OxiSelect™ Comet Assay Control Cells

CATALOG NUMBER: STA-354 **STORAGE:** Liquid Nitrogen

QUANTITY: Set of 2 vials; one treated and one untreated

Components

1. OxiSelect™ Comet Assay Control Cells, Untreated (Part No. 235401): One 0.5 mL vial of untreated, cryopreserved Jurkat T Cells at 1 x 10^5 cells/mL.
2. OxiSelect™ Comet Assay Control Cells, Treated (Part No. 235402): One 0.5 mL vial of Etoposide-treated, cryopreserved Jurkat T Cells at 1 x 10^5 cells/mL.

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.

The OxiSelect™ Comet Assay Control Cells are provided as a set; the set containing vials of healthy, untreated cells and DNA damaged, Etoposide-treated cells. These cells are intended for use as controls in the Cell Biolabs' OxiSelect™ Comet Assay under alkaline electrophoresis conditions.

Application

Cell Biolabs' OxiSelect™ Comet Assay Control Cells are designed for use in a single cell gel electrophoresis assay (SCGE) for simple evaluation of cellular DNA damage (under alkaline conditions). 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 alkaline 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). For a complete protocol, refer to Cell Biolabs' OxiSelect™ Comet Assay Kit insert (STA-350).

Related Products

1. STA-350: OxiSelect™ 3-Well Comet Assay Kit (5 Slides, 15 Assays)

2. STA-351: OxiSelect™ 3-Well Comet Assay Kit (25 Slides, 75 Assays)
3. STA-352: OxiSelect™ 3-Well Comet Assay Slides (5 Slides)
4. STA-353: OxiSelect™ 3-Well Comet Assay Slides (25 Slides)
5. STA-355: OxiSelect™ 96-Well Comet Assay Kit (1 Slide, 96 Assays)
6. STA-356: OxiSelect™ 96-Well Comet Assay Slide (1 Slide)

Storage

Upon receipt, store the vials in liquid nitrogen.

Aliquotting

1. Thaw the frozen cryovial of cells within 1–3 minutes by gentle agitation in a 37°C water bath. Decontaminate the cryovial by wiping the surface of the vial with 70% (v/v) ethanol.
2. Aliquot 50 µL of cell suspension into sterile cryovials and place cryovials immediately into a freezing container. Store overnight at -80 °C.
3. Transfer frozen vials to a -135 °C freezer or liquid nitrogen.

Comet Assay Preparation

1. Thaw frozen cell aliquots (above) within 1–3 minutes by gentle agitation in a 37°C water bath. Decontaminate the cryovial by wiping the surface of the vial with 70% (v/v) ethanol.
2. Add 1 mL of ice-cold PBS (without Mg^{2+} and Ca^{2+}) to the 50 µL aliquot.
3. Centrifuge cells at 700 x g for 2 minutes and aspirate all but 50 µL of the supernatant.
   Note: The cell pellet will not be visible. Be careful as to not remove the cell pellet.
4. Repeat steps 2-3 once more.
5. Continue with addition of Comet Assay Agarose (see Cell Biolabs' OxiSelect™ Comet Assay Kit insert STA-350).

Example of Results

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

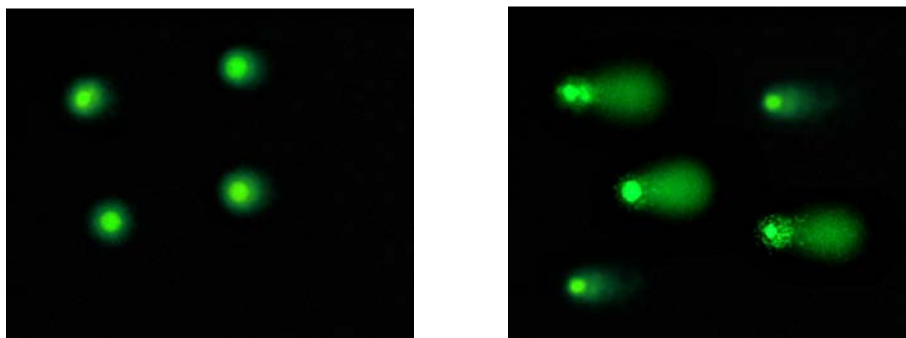
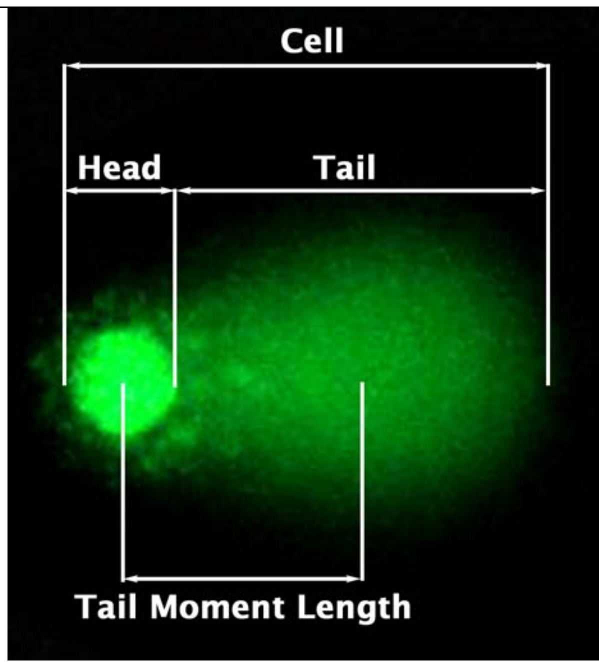


Figure 1. Etoposide Treatment of Jurkat Cells. Jurkat cells were untreated (left) or treated (right) with 20 µM Etoposide for 4 hours before performing Comet Assay (alkaline electrophoresis conditions, 33 V/300 mA for 15 minutes).

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 commonly 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 diagram on left)

A number of Comet analysis software programs are commercially available, such as LACAAS from Loats Associates, Inc.) and Comet Assay IV from Perceptive Instruments.

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

References

1. Ostling, O., and Johanson, K. J. (1984). Micro gel electrophoretic study of radiation induced DNA damages in individual mammalian cells. *Biochem. Biophys. Res. Commun.* **123,** 291–298.

2. Singh, N. P., McCoy, M. T., Tice, R. R., and Schneider, E. L. (1988). A simple technique for quantification of low levels of DNA damage in individual cells. *Exp. Cell. Res.* **175,** 184–191.

3. Olive, P. L., Banath, J. P., and Durand, R. E. (1990a). Heterogeneity in radiation induced DNA damage and repair in tumor and normal cells using the "Comet" assay. *Radiat. Res.* **122,** 86–94.

4. De Boeck, M., Touil, N., De Visscher, G., Vande, P. A., and Kirsch-Volders, M. (2000). Validation and implementation of an internal standard in Comet assay. *Mutat. Res.* **469,** 181–197.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND

BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2008-2013: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.