Product Manual

OxiSelect™ Alkaline Halo Assay Kit (3-Well Slides)

Catalog Number

STA-890 **15 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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.

A variety of methods have been developed for detecting DNA damage. 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.

Sestili and Cantoni (see Ref. 1) developed a novel technique, the alkaline-halo assay (AHA), which allows the measurement of DNA damage at the single-cell level. The AHA is based on the observation that osmotically driven radial diffusion of damaged DNA fragments through the pores of an agarose bed is an inverse function of the size of the DNA fragments. The term 'Halo' refers to the shape of radially diffused DNA fragments from isolated nuclei. The AHA presents some advantages with respect to the comet assay, it does not use electrophoresis to separate damaged DNA from undamaged DNA, but a short, post-lysis incubation in an alkaline hypotonic buffer. As a consequence, this method is simpler and more rapid than the comet assay, although the comet assay is still a more sensitive method for detecting DNA damage.

The OxiSelect™ Alkaline Halo Assay is a fast and sensitive kit for the measurement of cellular DNA damage. Each kit provides sufficient reagents to perform up to 15 assays.

Assay Principle

Cell Biolabs' OxiSelect™ Alkaline Halo Assay is a single cell-based DNA damage assay. First, individual cells are mixed with molten agarose before application to the OxiSelect™ Halo Assay Slide. These embedded cells are then treated with a Halo Diffusion buffer and alkaline solution. Under these conditions, the damaged DNA will osmotically diffuse into agarose gel to form a "halo" shape (see Figure 1). Nuclear diffusion factor (NDF) is determined for each assay sample. Each kit provides sufficient reagents to perform up to 15 assays.

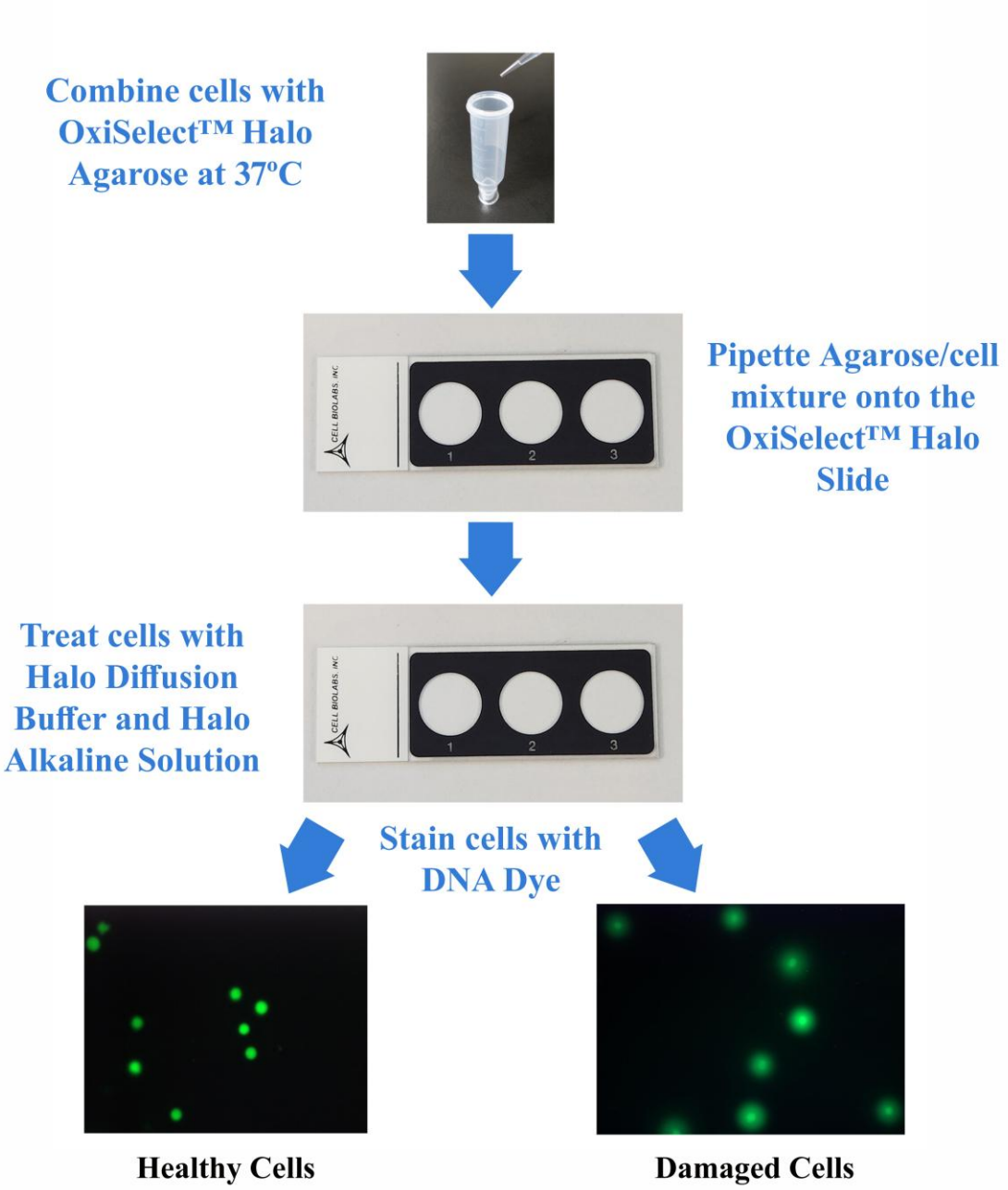


Figure 1: Alkaline Halo Assay Principle

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)

Kit Components (shipped at room temperature)

1. OxiSelect™ 3-Well Alkaline Halo Assay Slides (Part No. STA-892): Five slides.
2. OxiSelect™ Halo Agarose (Part No. 289001): One sterile 15 mL bottle.
3. 10X Halo Diffusion Buffer (Part No. 289002): One 20 mL bottle.
4. EDTA Solution, 500 mM (Part No. 235004): One 50 mL bottle.
5. 10X Halo Alkaline Solution (Part No. 289003): One 20 mL bottle.
6. Vista Green DNA Dye, 10000X (Part No. 235003): One 5 µL vial.

Materials Not Supplied

1. PBS (without Mg^{2+} and Ca^{2+})
2. NaCl powder
3. NaOH for pH adjustment
4. 70% Ethanol
5. TE Buffer (10 mM Tris, pH 7.5, 1 mM EDTA)

Storage

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

Preparation of Reagents

- OxiSelect™ Halo Agarose: Heat the Halo 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.
- 1X Halo Diffusion Buffer: To prepare 100 mL of 1X Halo Diffusion Buffer

NaCl	14.6 g
EDTA Solution (provided)	20.0 mL
10X Halo Diffusion Buffer (provided)	10.0 mL
DI H_2O	Adjust volume to 90 mL

Mix thoroughly to dissolve NaCl. Slowly adjust the Halo Diffusion Buffer to pH 10.0 with 10 N NaOH, then QS to 100 mL with DI H_2O. Chill 1X Halo Diffusion Buffer to 4ºC before use.

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

- 1X Halo Alkaline Solution: Prepare a 1X Halo Alkaline Solution by diluting the provided 10X stock 1:10 in DI H_2O. Chill 1X Halo Alkaline 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 1X Halo Diffusion Buffer and 1X Halo Alkaline Solution (see Preparation of Reagents) prior to performing the assay. Chill all solutions to 4ºC thoroughly.
2. Heat OxiSelect™ Halo 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 Halo Agarose (step 2) at 1:10 ratio (v/v), mix well by pipetting, and immediately transfer 75 µL/well onto the OxiSelect™ Halo Assay 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.

Assay Protocol

1. Carefully, transfer the slide to a small basin/container containing pre-chilled 1X Halo Diffusion Buffer (~25 mL/slide). Immerse the slide in the buffer for 30 minutes at 4ºC in the dark.
2. Carefully, aspirate the Halo Diffusion Buffer from the container and replace with pre-chilled 1X Halo Alkaline Solution (~25 mL/slide). Immerse the slide in the solution for 30 minutes at 4ºC in the dark.
3. Carefully, aspirate the Halo Alkaline Solution from the container and replace with 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 30-60 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™ Alkaline Halo Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

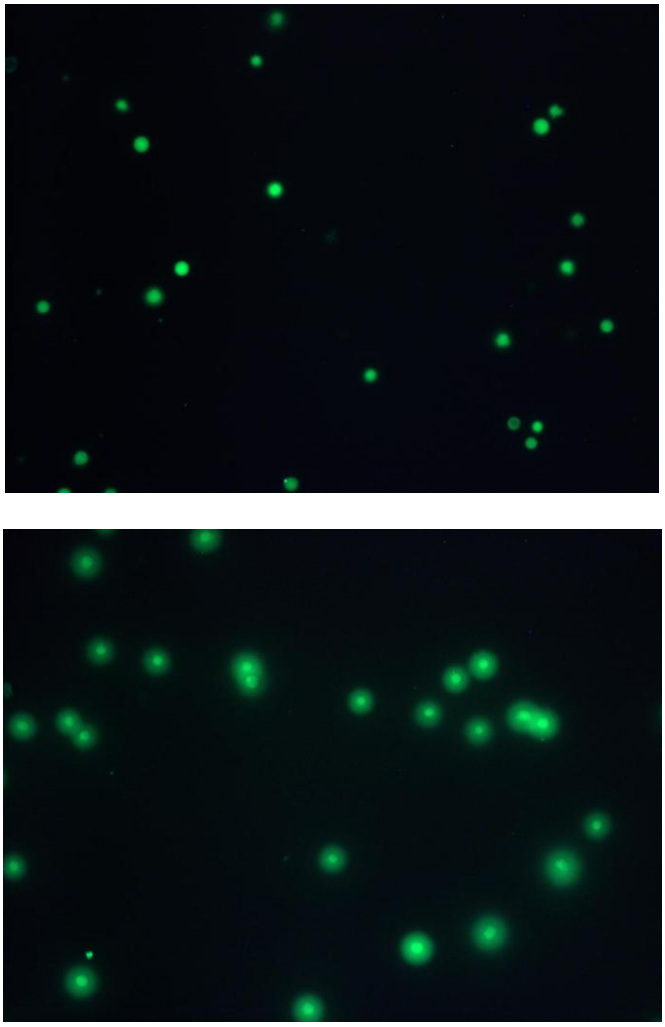
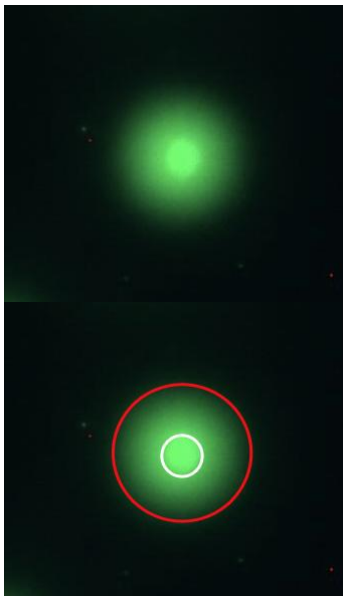


Figure 2. H_2O_2 Treatment of 293 Cells. 293 cells were untreated (top) or treated (bottom) with 1 mM H_2O_2 for 30 min before performing Alkaline Halo Assay.

Calculation of Results



The nuclear diffusion factor (NDF) is used as a quantitative measure of DNA damage in a halo assay. NDF represents the ratio of the total area (area within red line in Fig. 3 picture) of the halo area (diffused DNA) plus the nucleus area to the area of the nucleus alone (area within white line in Fig. 3). Higher NDF values indicate increased DNA damage, as more fragmented DNA diffuses further from the nucleus. At least 50 - 100 cells should be analyzed per sample.

$$\text{NDF} = \frac{\text{Total Area (Area of Halo + Area of Nucleus)}}{\text{Area of Nucleus}}$$

A number of Halo Assay analysis software programs are commercially available, such as and Image J and Halo J.

Figure 3: Typical Damaged DNA in Alkaline Halo Assay.

References

1. Sestili P., and Cantoni O. (1999) *Free Radic. Biol. Med.* **26**, 1019-1026.
2. Ostling, O., and Johanson, K. J. (1984). *Biochem. Biophys. Res. Commun.* **123,** 291–298.
3. Sestili P., Martinelli C., and Stocchi V. (2006) *Mutat Res* **607**, 205–214.

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.

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