Product Manual

OxiSelect™ DNA Double Strand Break (DSB) Staining Kit

Catalog Number

STA-321 100 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

DNA double-strand breaks (DSBs) are probably the most dangerous of the many different types of DNA damage that occur within the cell. DSBs are generated by exogenous agents such as ionizing radiation (IR) or by endogenously generated reactive oxygen species and occur as intermediates during meiotic and V(D)J recombination. A very early step in the cellular response to DSBs is the phosphorylation of a histone H2A variant, H2AX, at the sites of DNA damage. H2AX is rapidly phosphorylated (within seconds) at serine 139 when DSBs are introduced into mammalian cells resulting in discrete γ-H2AX (phosphorylated H2AX) foci at the DNA damage sites. H2AX phosphorylation also appears to be a general cellular response to processes involving DSB intermediates including V(D)J recombination in lymphoid cells and meiotic recombination in mice. Phosphorylation of H2A at serine 139 causes chromatin decondensation and appears to play a critical role in the recruitment of repair or damage-signaling factors to the sites of DNA damage.

Cell Biolabs' OxiSelect™ DNA DSB Staining Kit is based on the phosphorylation of the histone H2A.X at serine 139 in response to DNA damaging agents which cause double strand breaks in cells that are cultured in microtiter plates. The kit provides sufficient reagents for up to 100 stainings in 96-well plate.

Related Products

1. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
2. STA-324: OxiSelect™ Oxidative DNA Damage Quantitation Kit (AP sites)
3. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
4. STA-352: OxiSelect™ Comet Assay Slides (3-Well), 5 Slides
5. STA-355: OxiSelect™ 96-Well Comet Assay Kit

Kit Components (shipped on blue ice)

1. Anti-Phospho-Histone H2A.X (Ser 139) Antibody (100X) (Part No. 232101): One tube – 100 µL.
2. Secondary Antibody, FITC Conjugate (100X) (Part No. 232102): One amber tube – 100 µL.
3. DNA DSB Inducer (20 mM) (Part No. 232103): One tube – 50 µL of 20 mM Etoposide in DMSO.

Materials Not Supplied

1. Cell line of interest
2. 3.7% Formaldehyde in PBS
3. 90% Methanol
4. PBS
5. Blocking/Antibody Incubation Buffer (1% BSA/PBS)
6. Wash Buffer (PBS containing 0.05% Tween-20)

Storage

Store all kit components at -20ºC.

Preparation of Reagents

- 1X Anti-Phospho-Histone H2A.X Antibody Solution: Prepare a 1X Anti-Phospho-Histone Antibody Solution by diluting the provided 100X Anti-Phospho-Histone Antibody stock 1:100 in 1% BSA/PBS. Use the working solution immediately.
- 1X Secondary Antibody, FITC Conjugate Solution: Prepare a 1X Secondary Antibody Solution by diluting the provided 100X stock 1:100 in 1% BSA/PBS. Use the working solution immediately.
- DNA DSB Inducer: Dilute Etoposide a minimum of 1:200 in culture medium. Vortex to homogeneity. Use the working solution immediately.
- 90% Methanol: Dilute 100% Methanol to 90% with DI H_2O (9:1 ratio). Store the solution at -20ºC.

Assay Protocol

The following assay protocol is written for a 96-well format. Refer to the below table for the appropriate dispensing volumes of other plate formats.

Note: using other plate formats will decrease the number of assays possible with this kit.

	96-well	48-well	24-well	12-well	6-well
3.7% Formaldehyde/PBS (µL/well)	100	200	400	800	1600
90% Methanol (µL/well)	100	200	400	800	1600
Wash Buffer (µL/well)	200	400	800	1500	3000
Blocking Buffer (µL/well)	200	400	800	1500	3000
1X Anti-Phospho-Histone Antibody Solution (µL/well)	100	200	400	800	1600
1X Secondary Antibody, FITC Conjugate Solution (µL/well)	100	200	400	800	1600

Table 1. Dispensing Volumes of Different Plate Formats.

I. Cell Seeding

1. Harvest and resuspend cells in culture medium at 5 x 10^5 cells/mL. Seed 100 µL in each well of a 96-well plate and incubate overnight at 37ºC, 5% CO_2 (cells should be > 80% confluent).
2. (Optional) Aspirate the culture medium and add 100 µL/well of diluted DNA DSB Inducer, or desired DSB agent, and incubate for 1 hour at 37ºC, 5% CO_2.

II. Immunofluorescence Staining

1. Carefully remove medium from the wells by tilting the plate and aspirating from the edge. Fix the cells by gently adding 100 µL of 3.7% Formaldehyde/PBS to each well of the 96-well plate, taking care not to dislodge the cells. Incubate 10 minutes at room temperature.
2. Gently wash the fixed cells once with 200 µL of 1X PBS.
3. Aspirate the wells and add 100 µL of ice-cold 90% Methanol to each well. Incubate 10 minutes at 4ºC.
4. Gently wash the fixed cells once with 200 µL of 1X PBS.
5. Aspirate the wells and add 200 µL of Blocking Buffer (see Materials Not Supplied section) to each well. Incubate for 30 minutes at room temperature on an orbital shaker.
6. Aspirate the wells and add 100 µL of 1X Anti-Phospho-Histone Antibody Solution (see Preparation of Reagents section) to each well. Incubate for 1 hour at room temperature on an orbital shaker.

7. Gently wash the wells 5 times with 200 µL Wash Buffer (PBST).
8. Aspirate the wells and add 100 µL of 1X Secondary Antibody, FITC Conjugate Solution (see Preparation of Reagents section) to each well. Incubate for 1 hour at room temperature on an orbital shaker.
9. Gently wash the wells 5 times with 200 µL Wash Buffer (PBST).
10. Aspirate and add 200 µL 1X PBS to each well.
11. View staining with a fluorescence microscope using FITC filter.

Example of Results

The following figure demonstrates typical phospho-Histone 2A.X staining results. One should use the data below for reference only. This data should not be used to interpret actual results.

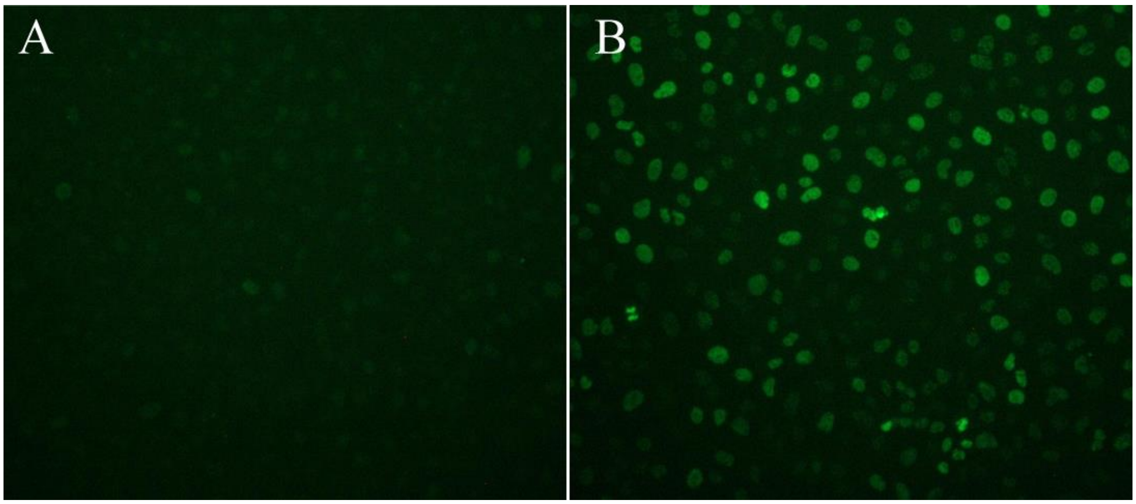

Figure 1: DNA DSB Formation in A549 Cells. A549 Cells were seeded at 50K/well overnight, then treated with (right) and without (left) 100 µM Etoposide for 1 hour. Immunofluorescence staining was performed as described in the Assay Instructions.

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Recent Product Citations

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