Version 1 Last updated 12 December 2018

ab242296 Gamma H2A.X Staining Kit

View Gamma H2A.X Staining Kit datasheet: www.abcam.com/ab242296

For the quantitative measurement detection of histone H2A.X phosphorylation.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Gamma H2A.X Staining Kit (ab242296) 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.

2. Protocol Summary

Harvest cells and resuspend in culture medium to 5 x 10^5 cell/mL. Add 100 µL of cell suspension to each well. Incubate overnight at 37°C/5% CO_2 (cell should be at >80% confluent).

[Optionally, aspirate medium and add 100 µL of diluted DNA DSB Inducer to selected wells and incubate for 1 hour at 37°C/5% CO_2.]

Remove medium. Fix cells with 100 µL of 3.7% Formaldehyde/PBS.

Wash cells with 200 µL PBS. Add 100 µL ice-cold 90% methanol.

Wash cells with 200 µL PBS. Add 200 µL of Blocking Buffer. Incubate for 30 minutes at room temperature.

Aspirate the wells and add 100 µL of 1X Anti-Phospho-Histone H2A.X (Ser139) Antibody Solution. Incubate for 1 hour at room temperature.

Wash the wells 5 times with 200 µL Wash Buffer (PBST).

Add 100 µL of 1X Secondary Antibody, FITC Conjugate Solution). Incubate for 1 hour at room temperature.

Wash the wells 5 times with 200 µL Wash Buffer (PBST). Add 200µL PBS

View staining with fluorescent microscope using FITC filter.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
Anti-Phospho-Histone H2A.X (Ser139) Antibody	100 µL	-20°C
DNA DSB Inducer	50 µL	-20°C
Secondary Antibody, FITC-Conjugate	100 µL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Cell line of interest
- 3.7% formaldehyde in PBS
- 90% methanol
- PBS
- Blocking/Antibody Incubation Buffer (1% BSA/PBS)
- Wash Buffer (PBS containing 0.05% Tween-20)

  Fluorescence microscope with FITC filter

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 1X Anti-Phospho-Histone H2A.X (Ser139) Antibody:

6.1.1 Prepare a 1X Anti-Phospho-Histone Antibody Solution by diluting the provided 100X Anti-Phospho-Histone Antibody stock 1:100 in 1% BSA/PBS.

6.1.2 Use the working solution immediately.

6.2 1X Secondary Antibody, FITC-Conjugate:

6.2.1 Prepare a 1X Secondary Antibody Solution by diluting the provided 100X stock 1:100 in 1% BSA/PBS.

6.2.2 Use the working solution immediately.

6.3 DNA DSB Inducer:

6.3.1 Dilute Etoposide a minimum of 1:200 in culture medium. Vortex to homogeneity.

6.3.2 Use the working solution immediately.

7. Sample Preparation

Cell seeding:

7.1 Harvest and resuspend cells in culture medium at 5 x 105 cells/mL. Seed 100 µL in each well of a 96-well plate and incubate overnight at 37°C, 5% CO2 (cells should be > 80% confluent).

7.2 (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 37°C, 5% CO2.

8. Assay Procedure

– We recommend that you assay all controls and samples in duplicate.

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.

Δ 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	4600
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

8.1 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 for 10 minutes at room temperature.

8.2 Gently wash the fixed cells once with 200 µL of 1X PBS.

8.3 Aspirate the wells and add 100 µL of ice-cold 90% Methanol to each well. Incubate 10 minutes at 4°C.

8.4 Gently wash the fixed cells once with 200 µL of 1X PBS.

8.5 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.

8.6 Aspirate the wells and add 100 µL of 1X Anti-Phospho-Histone H2A.X (Ser139) Antibody Solution (see Preparation of Reagents section) to each well. Incubate for 1 hour at room temperature on an orbital shaker.

8.7 Gently wash the wells 5 times with 200 µL Wash Buffer (PBST).

8.8 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.

8.9 Gently wash the wells 5 times with 200 µL Wash Buffer (PBST).

8.10 Aspirate and add 200 µL 1X PBS to each well.

8.11 View staining with a fluorescence microscope using FITC filter.

9. Data Analysis

9.1 View staining with a fluorescence microscope using FITC filter. Cells positive for phosphorylated Gamma H2A.X are stained green (see Typical Data, below).

10. Typical Data

Typical standard curve - data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

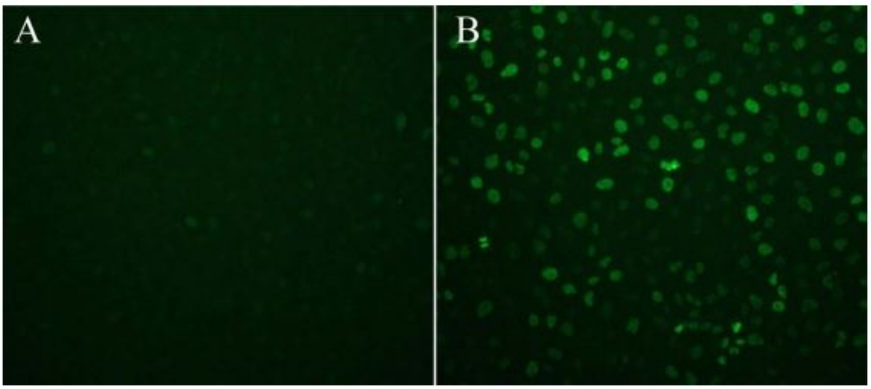

Figure 1. DNA DSB Formation in A549 cells. A549 cells were seeded at 50,000/well overnight, then treated with (B) and without (A) 100 µM Etoposide for 1 hour.

11. Notes

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