γH2AX DNA Damage Detection Kit (Mouse mAb/Green)

Introduction

DNA damage refers to the disruption or alteration of the DNA molecule structure, which can be caused by various factors, including ultraviolet radiation, ionizing radiation, genotoxic chemicals, and chemotherapeutic drugs. DNA damage takes many forms, including base modifications, intrastrand and interstrand crosslinks, DNA single-strand breaks, and DNA double-strand breaks (DSBs). Among these, DSBs are considered the most severe form of DNA damage, potentially leading to genomic instability, triggering tumors, and other related diseases.

γ-H2AX is a biomarker that rapidly appears after DNA double-strand breaks. It is one of the variants of histone H2A. When DNA double-strand breaks occur, certain kinases such as ATM and ATR phosphorylate serine at the 139th position of H2AX, forming phosphorylated H2AX, i.e., γ-H2AX. The level of γ-H2AX can reflect the degree of DNA damage and repair, making it an important marker for studying DNA damage and apoptosis.

This kit uses the γ-H2AX immunofluorescence method to detect DNA damage. During detection, the cell nucleus is stained blue with DAPI, while the DNA damage area, due to the presence of γ-H2AX, exhibits green fluorescence, thereby visually displaying the location and extent of DNA damage. The γ-H2AX Mouse mAb provided in this kit can recognize γ-H2AX in humans, mice, and rats, allowing this kit to detect DNA damage in human, mouse, or rat cells or tissues through fluorescence microscopy or high-content screening.

Components and Storage

Size / Components	1 Kit	Storage
Fixation Solution	50 mL	-20°C
Wash Buffer	500 mL	4°C
Blocking Buffer	50 mL	4°C
γ-H2AX Mouse mAb	5 mL	4°C
Anti-Mouse 488	5 mL	4°C away from light
DAPI	50 mL	-20°C away from light
Mounting Medium	10 mL	4°C
Shipping: Blue ice	**Shelf life: 6 months**	

Protocol

1. **For adherent cells:**

1) Remove the culture medium and wash once with PBS.

2) Add Fixation Solution and fix for 5-15 minutes. The amount of Fixation Solution should be sufficient to cover the sample, typically adding 1 mL per well in a 6-well plate.

3) Remove the Fixation Solution and wash three times with Wash Buffer, each for 3-5 minutes. After the last wash, try to remove as much Wash Buffer as possible.

4) Add Blocking Buffer and block at room temperature for 10-20 minutes. The amount of Blocking Buffer should be sufficient to cover the sample, typically adding 1 mL per well in a 6-well plate.

5) Remove the Blocking Buffer and add γ-H2AX Mouse mAb, incubate at room temperature for 1 hour or overnight at 4ºC. The amount of γ-H2AX Mouse mAb should be sufficient to cover the sample, typically adding 1 mL per well in a 6-well plate.

6) Carefully transfer the mAb to an appropriate container, store at 4ºC.

***Note:** γ-H2AX Mouse mAb can generally be reused at least 5 times.

7) Add Wash Buffer and wash three times, each for 5-10 minutes.

8) Add Anti-Mouse 488 and incubate at room temperature for 1 hour. The amount of Anti-Mouse 488 should be sufficient to cover the sample, typically adding 1 mL per well in a 6-well plate.

9) Carefully transfer Anti-Mouse 488 to an appropriate container, store at 4ºC.

***Note:** Anti-Mouse 488 can generally be reused at least 5 times.

10) Add Wash Buffer and wash twice, each for 5-10 minutes.

11) Add DAPI and stain at room temperature for about 5 minutes. The amount of DAPI should be sufficient to cover the sample, typically adding 1 mL per well in a 6-well plate.

12) Remove the DAPI and wash three times with Wash Buffer, each for 3-5 minutes.

13) If using larger wells such as a 6-well plate, apply an appropriate amount of Mounting Medium, cover with a coverslip and observe under a fluorescence microscope. If using a 96-well plate, observe directly under the retained Wash Buffer. The γ-H2AX staining in the cell nucleus appears as green fluorescence (Ex/Em: 490/525 nm), and DAPI staining appears as blue fluorescence (Ex/Em: 350/470 nm).

2. **For suspension cells:**

1) Collect cells by centrifugation, wash once with PBS. After removing PBS, gently disperse the cells.

2) Add Fixation Solution, gently resuspend the cells, and fix for 5-15 minutes.

3) Centrifuge and remove the Fixation Solution. Add Wash Buffer and wash once.

4) Take a small amount of Wash Buffer to resuspend the cells, drop onto a coverslip or slide, and prepare a smear. After drying completely, continue with the subsequent steps.

5) Wash Buffer washes twice, each for 5 minutes.

6) Proceed to step 1.4-1.13 or adopt the drop staining operation, the specific steps are as follows:

7) Use an immunohistochemistry pen to circle and dry.

8) Drop an appropriate amount of Blocking Buffer to fully cover the sample without overflowing. Then incubate in a wet box for 10-20 minutes.

9) Aspirate the Blocking Buffer, drop an appropriate amount of γ-H2AX Mouse mAb to fully cover the sample, and incubate in a wet box at room temperature for 1 hour or overnight at 4ºC.

10) Aspirate the γ-H2AX Mouse mAb, wash three times with Wash Buffer, each for 5-10 minutes.

11) Drop Anti-Mouse 488 to fully cover the sample and incubate at room temperature for 1 hour.

12) Aspirate Anti-Mouse 488, wash 2-3 times with Wash Buffer, each for 5-10 minutes.

13) Drop DAPI to fully cover the sample and incubate at room temperature for 5 minutes.

14) Aspirate DAPI, wash 2-3 times with Wash Buffer, each for 5-10 minutes.

15) Drop an appropriate amount of Mounting Medium, cover with a coverslip and observe under a fluorescence microscope. The γ-H2AX staining in the cell nucleus appears as green fluorescence (Ex/Em: 490/525 nm), and DAPI staining appears as blue fluorescence (Ex/Em: 350/470 nm).

3. **For tissue sections:**

1) For paraffin sections, follow the routine deparaffinization and hydration treatment. For frozen sections, you can proceed directly to the next steps.

2) Proceed to step 1.2-1.13 or adopt the drop staining operation, proceed to step 2.7-2.15.

Note

1. The Fixation Solution is potentially toxic. Appropriate protective measures should be taken during operation to avoid contact with skin or inhalation.
2. When performing immunofluorescence staining, it is recommended to recover and reuse the used γ-H2AX Mouse mAb and Anti-Mouse 488, which can generally be reused at least 5 times. If the solution appears turbid or precipitated, discontinue use.
3. For your safety and health, please wear lab coats and gloves during the experiment.

4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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