DNA Damage Detection Kit - γH2AX - Green
DNA Damage Detection Kit - γH2AX - Red
DNA Damage Detection Kit - γH2AX - Deep Red

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/G265, G266, G267.pdf

General Information

H2AX is phosphorylated rapidly and widely upon DNA double-strand breaks. Among H2AX histone variants, H2AX with phosphorylated Ser-139 is called γH2AX. As a DNA damage marker, γH2AX is monitored to investigate genotoxicity and carcinogenicity of chemicals. Recent studies suggest that γH2AX is a marker for cellular senescence. The DNA Damage Detection Kit includes all necessary components, an anti-γH2AX antibody, secondary antibody (fluorophore labeled), and blocking solution for staining which allows ready-to-use immunodetection of γH2AX.

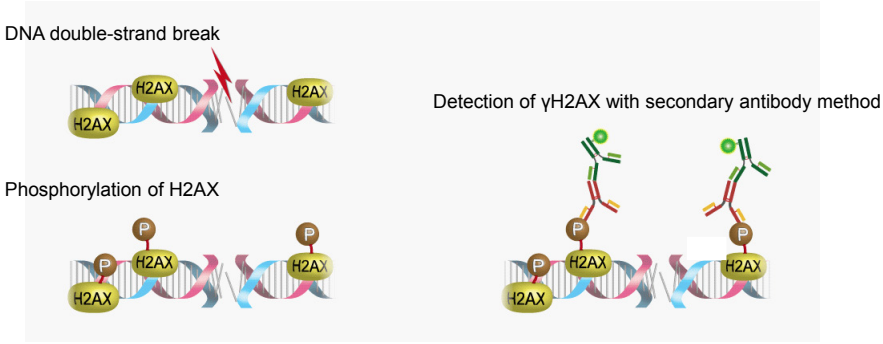

Figure 1. Detection of γH2AX

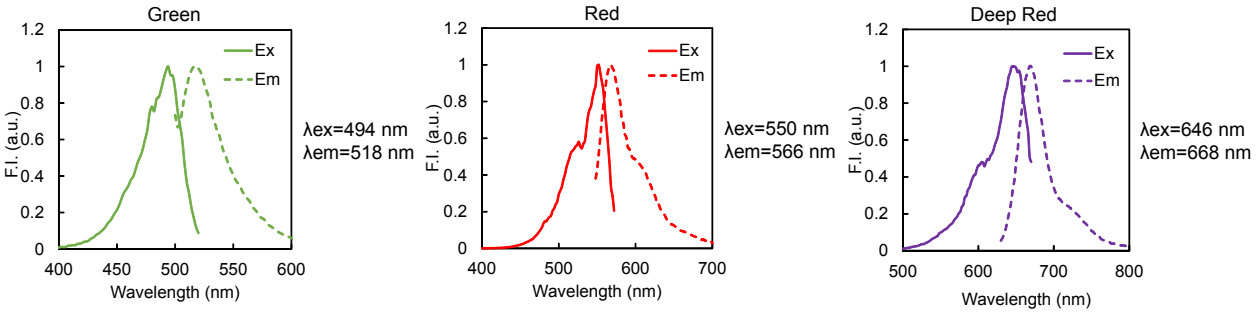

Figure 2. Excitation and emission spectra of fluorophores

Kit Contents

- Anti γH2AX antibody (Host ; Mouse)	x 1
※ The anti-γH2AX antibody used in the kit is manufactured by MAB Institute, Inc.	
- Secondary antibody - Green, Red or Deep Red (Host ; Goat)	x 1
- Blocking Solution	x 1

Storage Condition

Store at 0–5 °C

Required Equipment and Materials

- ddH_2O (double-deionized water)
- Phosphate Buffered Saline (PBS)
- Micropipette
- Paraformaldehyde (PFA)
- Triton-X
- 250 mM HEPES (pH7.4)

Preparation of Solutions

Preparation of the Anti γH2AX antibody stock solution

Add 100 µL ddH_2O to the Anti γH2AX antibody and dissolve by pipetting to prepare the Anti γH2AX antibody stock solution.
*Store the Anti γH2AX antibody stock solution at 0–5 °C .The prepared antibody solution is stable at 4°C for 3 weeks.

Preparation of the Anti γH2AX antibody staining solution

Dilute the Anti γH2AX antibody stock solution by 50 times with the Blocking Solution.
*Prepare working solution fresh each day.

Preparation of the Secondary antibody staining solution

Dilute the Secondary antibody - Green, Red or Deep Red 50 times with the Blocking Solution.
*Prepare the working solution fresh each day.

Below the table shows the number of staining possible with a kit.

Staining volume	Number of staining possible
100 µL	50
200 µL	25
2 mL	2

General Protocol

1. Seed cells on a dish, chamber slide or microplate for fluorescent imaging and culture overnight at 37°C in a 37°C incubator equilibrated with 95% air, 5% CO_2.
2. Discard the supernatant and wash the cells with PBS.
3. Remove the PBS and add 250 mmol/L HEPES (pH 7.4) containing 4% PFA and 0.1% Triton X-100 for fixation. Incubate the cells at room temperature for 5 minutes .
4. Discard the supernatant and wash the cells with PBS twice.
5. Remove the PBS and add PBS containing 1% Triton X-100. Incubate the cells at room temperature for 20 minutes.
6. Discard the supernatant and wash the cells with PBS twice.
7. Add the Blocking Solution and incubate the cells at room temperature for 20 minutes .
8. Discard the supernatant and wash the cells with PBS twice.
9. Remove the PBS and add the Anti γH2AX antibody staining solution. Incubate the cells at room temperature for 60 minutes .
10. Discard the supernatant and wash the cells with PBS twice.
11. Remove the PBS and add the Secondary antibody staining solution. Incubate the cells at room temperature for 60 minutes .
12. Discard the supernatant and wash the cells with PBS twice.
13. Observe the cells under a fluorescence microscope.

Usage Example

Detection of γH2AX in HeLa cells treated with doxorubicin

1. HeLa cells were seeded on a µ-slide 8 well (ibidi) and cultured overnight at 37°C in a 37°C incubator equilibrated with 95% air, 5% CO_2.
2. After discarding the supernatant, medium containing 0.5 µmol/L doxorubicin was added. The cells were incubated overnight at 37°C in the 37°C incubator equilibrated with 95% air, 5% CO_2.
3. After discarding the supernatant, the cells were washed with PBS.
4. The PBS was removed and 200 µL 250 mmol/L HEPES (pH 7.4) containing 4% PFA and 0.1% Triton X-100 was added for fixation. The cells were incubated at room temperature for 5 minutes.
5. After discarding the supernatant, the cells were washed with PBS twice.
6. The PBS was removed and 200 µL PBS containing 1% Triton X-100 was added. The cells were incubated at room temperature for 20 minutes.
7. After discarding the supernatant, the cells were washed with PBS twice.
8. The PBS was removed and 200 µL Blocking Solution was added. The cells were incubated at room temperature for 20 minutes.
9. After discarding the supernatant, the cells were washed with PBS twice.
10. The PBS was removed. The Anti γH2AX antibody staining solution was added. The cells were incubated at room temperature for 60 minutes.
11. After discarding the supernatant, the cells were washed with PBS twice.
12. The PBS was removed. The Secondary antibody staining solution was added. The cells were incubated at room temperature for 60 minutes.
13. After discarding the supernatant, the cells were washed with PBS twice.
14. The cells were observed under a fluorescence microscope.

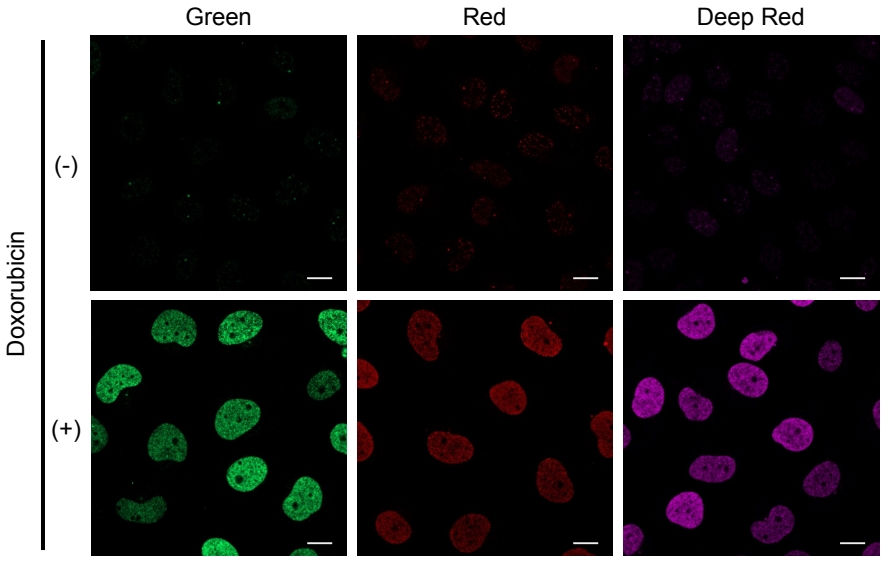

Figure 3. Detection of γH2AX in HeLa cells treated with doxorubicin

Green	Ex/Em = 488 nm/ 500–550 nm
Red	Ex/Em = 561 nm/ 570–620 nm
Deep Red	Ex/Em = 640 nm/ 650–700 nm
Scale bars	20µm

If you need more information, please contact Dojindo technical service.

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