
Product Manual

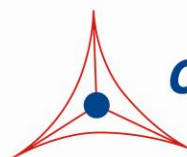
OxiSelect™ DNA Double Strand Break (DSB) Staining Kit, Trial Size

Catalog Number

STA-321-T

20 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. This Trial Size kit provides sufficient reagents for up to 20 stainings in a 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-350: OxiSelect™ Comet Assay Kit (3-Well Slides), 15 Assays
5. STA-351: OxiSelect™ Comet Assay Kit (3-Well Slides), 75 Assays
6. STA-352: OxiSelect™ Comet Assay Slides (3-Well), 5 Slides
7. STA-353: OxiSelect™ Comet Assay Slides (3-Well), 25 Slides
8. STA-355: OxiSelect™ 96-Well Comet Assay Kit
9. STA-356: OxiSelect™ 96-Well Comet Assay Slide

Kit Components

1. Anti-Phospho-Histone H2A.X (Ser 139) Antibody (100X) (Part No. 232101-T): One tube – 20 μ L.
2. Secondary Antibody, FITC Conjugate (100X) (Part No. 232102-T): One amber tube – 40 μ L.
3. DNA DSB Inducer (20 mM) (Part No. 232103): One tube – 50 μ L 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)
7. Fluorescence microscope with FITC filter

Storage

Store all kit components at -20°C.

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 H₂O (9:1 ratio). Store the solution at -20°C.

Assay Protocol

The following assay protocol is written for a 96-well format. Refer to the table below for the appropriate dispensing volumes for 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
3.7% Formaldehyde/PBS (μL/well)	100	200	400	800
90% Methanol (μL/well)	100	200	400	800
Wash Buffer (μL/well)	200	400	800	1500
Blocking Buffer(μL/well)	200	400	800	1500
1X Anti-Phospho-Histone Antibody Solution (μL/well)	100	200	400	800
1X Secondary Antibody, FITC Conjugate Solution (μL/well)	100	200	400	800

Table 1. Dispensing Volumes of Different Plate Formats.

I. Cell Seeding

1. Harvest and resuspend cells in culture medium at 5×10^5 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).
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.

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 μ L 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 μ L of 1X PBS.
3. Aspirate the wells and add 100 μ L of ice-cold 90% Methanol to each well. Incubate 10 minutes at 4°C.
4. Gently wash the fixed cells once with 200 μ L of 1X PBS.
5. Aspirate the wells and add 200 μ L 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 μ L 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 μ L Wash Buffer (PBST).
8. Aspirate the wells and add 100 μ L 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 μ L Wash Buffer (PBST).
10. Aspirate and add 200 μ L 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.

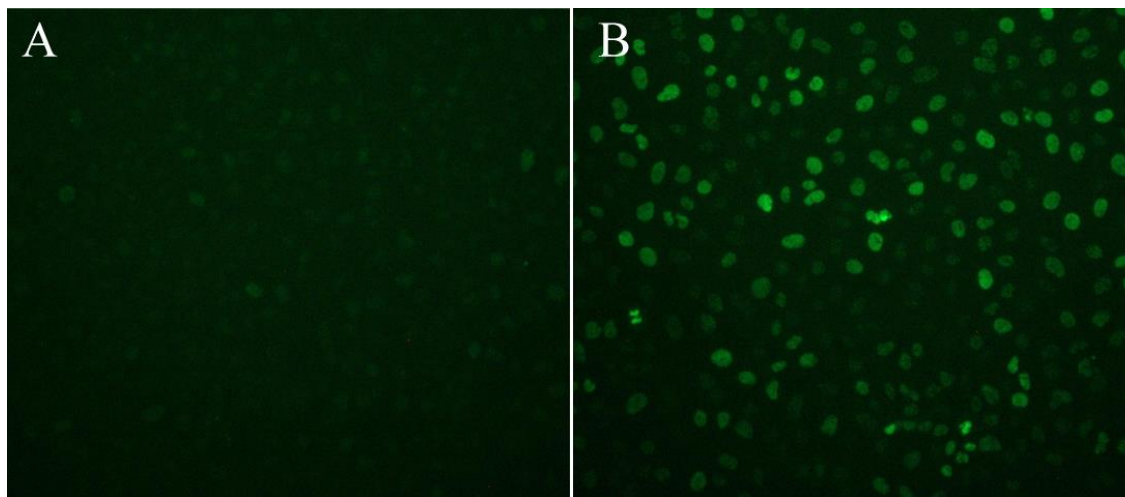


Figure 1: DNA DSB Formation in A549 Cells. A549 Cells were seeded at 50K/well overnight, then treated with (right) and without (left) 100 μ M Etoposide for 1 hour. Immunofluorescence staining was performed as described in the Assay Instructions.

References

1. Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S., and Bonner, W. M. (1998) *J. Biol. Chem.* **273**, 5858-5868.
2. Rogakou, E. P., Boon, C., Redon, C., and Bonner, W. M. (1999) *J. Cell Biol.* **146**, 905-915.
3. Paull, T. T., Rogakou, E. P., Yamazaki, V., Kirchgessner, C. U., Gellert, M., and Bonner, W. M. (2000) *Curr. Biol.* **10**, 886-895.
4. Chen, H. T., Bhandoola, A., Difilippantonio, M. J., Zhu, J., Brown, M. J., Tai, X., Rogakou, E. P., Brotz, T. M., Bonner, W. M., Ried, T., and Nussenzweig, A. (2000) *Science* **290**, 1962-1964.
5. Mahadevaiah, S. K., Turner, J. M. A., Baudat, F., Rogakou, E. P., de Boer, P., Blanco-Rodriguez, J., Jasin, M., Keeney, S., Bonner, W. M., and Burgoyne, P. S. (2001) *Nat. Genet.* **27**, 271-276.

Recent Product Citations

1. Takano, S. et al. (2023). Optimal timing of a γ H2AX analysis to predict cellular lethal damage in cultured tumor cell lines after exposure to diagnostic and therapeutic radiation doses. *J Radiat Res.* **64**(2):317-327. doi: 10.1093/jrr/rrac096.
2. Schlegel, J. et al. (2022). Biosensor Cell-Fit-HD4D for correlation of single-cell fate and microscale energy deposition in complex ion beams. *STAR Protoc.* **3**(4):101798. doi: 10.1016/j.xpro.2022.101798.
3. Douiev, L. et al. (2022). Replicative Stress Coincides with Impaired Nuclear DNA Damage Response in COX4-1 Deficiency. *Int J Mol Sci.* **23**(8):4149. doi: 10.3390/ijms23084149.

4. Niklas, M. et al. (2022). Biosensor for deconvolution of individual cell fate in response to ion beam irradiation. *Cell Rep Methods*. doi: 10.1016/j.crmeth.2022.100169.
5. Liu, Q. et al. (2021). Modular Assembly of Tumor-Penetrating and Oligomeric Nanozyme Based on Intrinsically Self-Assembling Protein Nanocages. *Adv Mater*. doi: 10.1002/adma.202103128.
6. Tessonier, T. et al. (2021). FLASH dose-rate helium ion beams: first in vitro investigations. *Int J Radiat Oncol Biol Phys*. doi: 10.1016/j.ijrobp.2021.07.1703.
7. Ekuban, A. et al. (2021). Role of Macrophages in Cytotoxicity, Reactive Oxygen Species Production and DNA Damage in 1,2-Dichloropropane-Exposed Human Cholangiocytes In Vitro. *Toxics*. **9**(6):128. Doi: 10.3390/toxics9060128.
8. Park, J.Y. et al. (2020). Targeted Therapy of Hepatocellular Carcinoma Using Gemcitabine-Incorporated GPC3 Aptamer. *Pharmaceutics*. **12**(10):E985. doi: 10.3390/pharmaceutics12100985.
9. Zhong, C. et al. (2020). Mechanism for enhanced transduction of hematopoietic cells by recombinant adeno-associated virus serotype 6 vectors. *FASEB J*. doi: 10.1096/fj.201902875r.
10. Yuan, Y. et al. (2020). Deterioration of hematopoietic autophagy is linked to osteoporosis. *Aging Cell*. doi: 10.1111/accel.13114.
11. Han, S. et al. (2019). Secretome analysis of patient-derived GBM tumor spheres identifies midkine as a potent therapeutic target. *Exp Mol Med*. **51**(12):147. doi: 10.1038/s12276-019-0351-y.
12. Zhang, Y. et al. (2019). N-Acetyl Cysteine as a Novel Polymethyl Methacrylate Resin Component: Protection against Cell Apoptosis and Genotoxicity. *Oxidative Medicine and Cellular Longevity*. doi: 10.1155/2019/1301736.
13. Wang, Z. et al. (2019). Changes in the Proliferation Rate, Clonogenicity, and Radiosensitivity of Cultured Cells During and After Continuous Low-Dose-Rate Irradiation. *Dose Response*. **17**(2):1559325819842733. doi: 10.1177/1559325819842733.
14. Izumi, Y. et al. (2019). Suplatast tosilate reduces radiation-induced lung injury in mice through suppression of oxidative stress. *Free Radic Biol Med*. **136**:52-59. doi: 10.1016/j.freeradbiomed.2019.03.024.
15. Nakade, S. et al. (2018). Biased genome editing using the local accumulation of DSB repair molecules system. *Nat Commun*. **9**(1):3270. doi: 10.1038/s41467-018-05773-6.
16. Douiev, L. et al. (2018). The pathomechanism of cytochrome c oxidase deficiency includes nuclear DNA damage. *Biochim Biophys Acta Bioenerg*. **1859**(9):893-900. doi: 10.1016/j.bbabi.2018.06.004.
17. Douiev, L. et al. (2018). Cytochrome c oxidase deficiency, oxidative stress, possible antioxidant therapy and link to nuclear DNA damage. *Eur J Hum Genet*. **26**(4):579-581. doi: 10.1038/s41431-017-0047-5.
18. Shirasugi, M. et al. (2016). Normal human gingival fibroblasts undergo cytoysis and apoptosis after long-term exposure to butyric acid. *Biochem. Biophys. Res. Commun*. doi: 10.1016/j.bbrc.2016.11.168.
19. Wu, S. T. et al. (2016). Cellular effects induced by 17- β -estradiol to reduce the survival of renal cell carcinoma cells. *J Biomed Sci*. **23**:67.
20. Cheng, K. P. et al. (2016). Blue light modulates murine microglial gene expression in the absence of optogenetic protein expression. *Sci Rep*. doi:10.1038/srep21172.
21. Ohashi, S. et al. (2014). Preclinical validation of talaporfin sodium-mediated photodynamic therapy for esophageal squamous cell carcinoma. *PLoS One*. **9**:e103126.
22. Dokic, I. et al. (2014). High resistance to X-rays and therapeutic carbon ions in glioblastoma cells bearing dysfunctional ATM associates with intrinsic chromosomal instability. *Int J Radiat Biol*. **91**:157-165.

23. Matsuda, S. et al. (2014). An easy-to-use genotoxicity assay using EGFP-MDC1-expressing human cells. *Gene Environ.* **36**:17-28.
24. Zhuge, C.C. et al. (2014). Fullerenol protects retinal pigment epithelial cells from oxidative stress–induced premature senescence via activating SIRT1. *Invest Ophthalmol Vis Sci.* **55**:4628-4638.

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

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