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# Datasheet for ABIN2344964 OxiSelect<sup>™</sup> DNA Double Strand Break (DSB) Staining Kit

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Publications

6



### Overview

Quantity:	20 tests
Reactivity:	Others
Application:	Biochemical Assay (BCA)

# Product Details

Brand:	OxiSelect™
Sample Type:	Cell Samples
Detection Method:	Fluorometric
Characteristics:	OxiSelect <sup>™</sup> 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.
Components:	1. Anti-Phospho-Histone H2A.X (Ser 139) Antibody (100X) : One tube - 20 μL. 2. Secondary Antibody, FITC Conjugate (100X) : One amber tube - 40 μL. 3. DNA DSB Inducer (20 mM) : One tube - 50 μL of 20 mM Etoposide in methanol. 2
Material not included:	<ol> <li>Cell line of interest</li> <li>3.7 % Formaldehyde in PBS</li> <li>90 % Methanol</li> <li>PBS</li> <li>Blocking/Antibody Incubation Buffer (1 % BSA/PBS)</li> <li>Wash Buffer (PBS containing 0.05 % Tween-20)</li> <li>Fluorescence microscope with FITC filter</li> </ol>

## Target Details

#### Background:

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.

# Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	Detects phosphorylation of histone H2A.X
	See immunofluorescence in about 3 hours
	DNA double-strand break inducer included
Reagent Preparation:	• 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.
	<ul> <li>90 % Methanol: Dilute 100 % Methanol to 90 % with DI H2O (9:1 ratio). Store the solution at - 20 °C.</li> </ul>
Assay Procedure:	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 100 200 400 800 (µL/well) 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 100 200 400 800 Antibody Solution ( $\mu$ L/well) 1X Secondary Antibody, FITC
	Conjugate 100 200 400 800 Solution (µL/well) Table

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	1. Dispensing Volumes of Different Plate Formats. 3
	<ol> <li>Cell Seeding</li> <li>Harvest and resuspend cells in culture medium at 5 x 105 cells/mL. Seed 100 μL in each</li> </ol>
	well of a 96-well plate and incubate overnight at 37 °C, 5 % CO2 (cells should be > 80 % confluent).
	<ol> <li>(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.</li> </ol>
	II. Immunofluorescence Staining
	<ol> <li>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.</li> </ol>
	2. Gently wash the fixed cells once with 200 $\mu$ L of 1X PBS.
	<ol> <li>Aspirate the wells and add 100 μL of ice-cold 90 % Methanol to each well. Incubate</li> <li>10 minutes at 4 °C.</li> </ol>
	4. Gently wash the fixed cells once with 200 $\mu L$ of 1X PBS.
	5. Aspirate the wells and add 200 $\mu L$ 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 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 $\mu$ L 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 $\mu$ L Wash Buffer (PBST).
	10. Aspirate and add 200 $\mu L$ 1X PBS to each well.
	11. View staining with a fluorescence microscope using FITC filter. 4
Restrictions:	For Research Use only
Handling	
Storage:	-20 °C
Storage Comment:	Store all kit components at -20°C.
Publications	
Product cited in:	Wu, Ku, Huang, Wang, Lin, Chen: "Cellular effects induced by 17- $\beta$ -estradiol to reduce the
	survival of renal cell carcinoma cells." in: <b>Journal of biomedical science</b> , Vol. 23, Issue 1, pp. 67, (2016) (PubMed).
	Shirasugi, Nishioka, Yamamoto, Nakaya, Kanamura: "Normal human gingival fibroblasts

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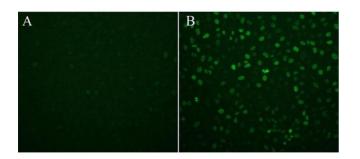
Dokic, Mairani, Brons, Schoell, Jauch, Krunic, Debus, Régnier-Vigouroux, Weber: "High resistance to X-rays and therapeutic carbon ions in glioblastoma cells bearing dysfunctional ATM associates with intrinsic chromosomal instability." in: **International journal of radiation biology**, Vol. 91, Issue 2, pp. 157-65, (2015) (PubMed).

Zhuge, Xu, Zhang, Li, Li, Li, Chen, Liu, Shang, Xu, Lu, Wang, Lu, Xu: "Fullerenol protects retinal pigment epithelial cells from oxidative stress-induced premature senescence via activating SIRT1." in: **Investigative ophthalmology & visual science**, Vol. 55, Issue 7, pp. 4628-38, (2014) (PubMed).

Ohashi, Kikuchi, Tsurumaki, Nakai, Kasai, Horimatsu, Miyamoto, Shimizu, Chiba, Muto: " Preclinical validation of talaporfin sodium-mediated photodynamic therapy for esophageal squamous cell carcinoma." in: **PLoS ONE**, Vol. 9, Issue 8, pp. e103126, (2014) (PubMed).

There are more publications referencing this product on: Product page

### Images



#### Immunofluorescence

Image 1. DNA Double-Strand Break Formation in A549
Cells. A549 cells were seeded at 50,000 cells/well overnight.
(A) Untreated cells. (B) Cells treated with 100 μM Etoposide
for 1 hour. Immunofluorescence staining was then
performed according to the Assay Protocol.