# DNA Damage Assay (Fluorescent)

## quick and easy cell-based assay for the DNA damage marker, phospho-H2AX

Active Motif's DNA Damage Assay is a simple, fast and accurate 2-color fluorescent assay for the induction of double-stranded DNA breaks. When cell cultures or animals are exposed to ionizing radiation or certain treatment compounds, double-stranded DNA breaks are created that rapidly result in phosphorylation of the histone variant H2AX at serine 139. Because phosphorylation of H2AX at Ser139 correlates very closely with each double-stranded DNA break, phospho-H2AX is a sensitive marker for assessing the effects of DNA-damage and repair agents. For more complete information on this and other related assays, please visit us at www.activemotif.com.

## A simple fluorescent assay to measure a treatment's effect on double-stranded DNA breaks

The DNA Damage Assay utilizes the highly specific DNA damage marker antibody, Histone H2AX phospho Ser139 pAb. Cells are grown and treated in a 96-well plate, then fixed and incubated with the phospho-H2AX antibody, which is subsequently detected with the Chromeo™ 488-labeled secondary antibody. The cells are then washed and stained with propidium iodide, and the plate is read on a fluorescent plate reader.

This assay is suited for high-throughput and high-content fluorescent scanning applications (Figure 1). Each kit contains reagents for two full 96-well plates including apoptosis marker antibody, propidium iodide and the positive control treatment compound, etoposide, which generates a high DNA-damage reference population (Figure 2).

- **Easy** – very little hands-on-time
- **Accurate** – Chromeo 488 versus propidium iodide readout to measure treatment effects
- **Specific** – High-quality antibodies ensure no problems with background

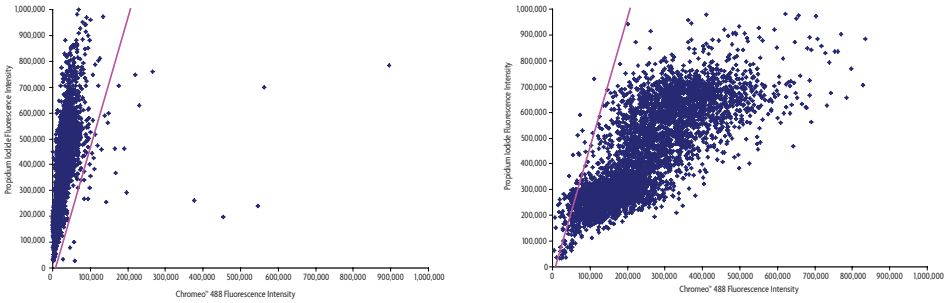


**Figure 1: Single cell analysis of control and etoposide-treated HeLa cells.**
Control HeLa cells (left) and etoposide-treated HeLa cells (right) analyzed with the Blueshift IsoCyte™. The shift in the cell population to the right of the dividing line in the etoposide-treated cells indicates a high degree of DNA damage in these cells.

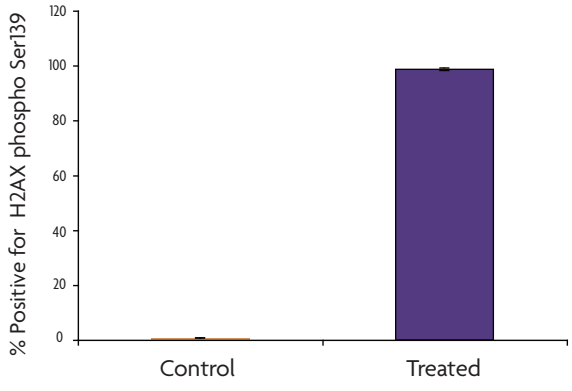


**Figure 2: Percent of HeLa cells positive for phospho-H2AX.**
Levels of phospho-H2AX measured in control and etoposide-treated cells. Averages of quadruplicate wells are shown.

### CONTENTS & STORAGE

H2AX phospho Ser139 pAb, Chromeo™ 488 Goat anti-Rabbit IgG, propidium iodide, etoposide and RNase A. Reagents are to be stored at -20°C. All reagents are guaranteed stable for 6 months from date of receipt when stored properly.

| Product | Format | Catalog No. |
|---|---|---|
| DNA Damage Assay (Fluorescent) | 2 x 96 rxns | 18030 |