

## Quick Reference Card

# Muse® Multi-Color DNA Damage Kit

## MCH200107

To investigate the DNA damage response through the ATM dependent signaling pathway by simultaneous measurement of phosphorylated ATM and H2A.X in any given cell population  
For Research Use Only. Not for use in diagnostic procedures.

### Storage Conditions

All reagents must be stored at 2–8°C.

### Materials Recommended

- Guava® Muse® Cell Analyzer
- Test tubes for sample prep and storage
- Tissue culture reagents, i.e., HBSS, PBS w/o  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ , cell dislodging buffers, etc.
- Micropipettes and tips
- Table top centrifuge
- Mechanical vortex
- Deionized water
- Cells of interest in suspension

- Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Catalogue No. 16466-030, or equivalent)

### Kit Components

- 20X Anti-phospho-ATM (Ser1981), PE: (Part No. CS208162), 250  $\mu\text{L}$
- 20X Anti-phospho-Histone H2A.X (Ser139), PECy5: (Part No. CS208174), 250  $\mu\text{L}$
- 5X Assay Buffer: (Part No. CS202124), 55 mL
- Fixation Buffer: (Part No. CS202122), 13 mL
- 1X Permeabilization Buffer: (Part No. CS203284), 14 mL

### Assay Protocol

Prepare cell cultures for experimentation (treated or untreated). → Centrifuge cells at 300 xg for 5 min and wash once with 1X PBS. → Fix cells in Fixation Buffer for 10 min on ice, followed by a wash step. → Permeabilize cells with Permeabilization Buffer for 10 min on ice, followed by a wash step. → Add 200,000 cells to each tube (treated or untreated).

→ Add 10  $\mu\text{L}$  of antibody cocktail to 90  $\mu\text{L}$  of 1X Assay Buffer per tube/test. Incubate for 30 min at room temp (dark). → Centrifuge cells at 300 xg for 5 min and wash with 1X Assay Buffer. → Resuspend in 200  $\mu\text{L}$  1X Assay Buffer. → Acquire samples on Muse® Cell Analyzer



## Expected Results

**Figures A and B.** HeLa cells were exposed to 10  $\mu\text{M}$  Etoposide for 24 hours to induce DNA damage, and then stained with both anti-phospho-Histone H2A.X (Ser139) and anti-phospho-ATM (Ser1981) antibodies in multiplex. Samples were acquired using the Guava® Muse® Cell Analyzer and statistical results are shown here. Figure A shows the results summary, while Figure B shows results displayed in dot plot format.

The statistics captured in this assay show the relative percentages for each population as it is calculated within the total cell population. Cells which express ATM, H2A.X, or both can be seen by the data on upper left, lower right, and upper right quadrants of the dot plot, respectively. In this cell population, 64.5% shows co-activation of ATM and H2A.X upon treatment, indicating DNA damage and presence of double-strand breaks.

For more information, refer to the comprehensive kit user's guide for this product, which can be found at [www.luminexcorp.com/flowkits](http://www.luminexcorp.com/flowkits) (search for Catalogue Code MCH200107).

## Related Products

For research use only. Not for use in diagnostic procedures.

Muse® EGFR-RTK Activation Dual Detection Kit - MCH200102

Muse® PI3K Activation Dual Detection Kit - MCH200103

Muse® H2A.X Activation Dual Detection Kit - MCH200101

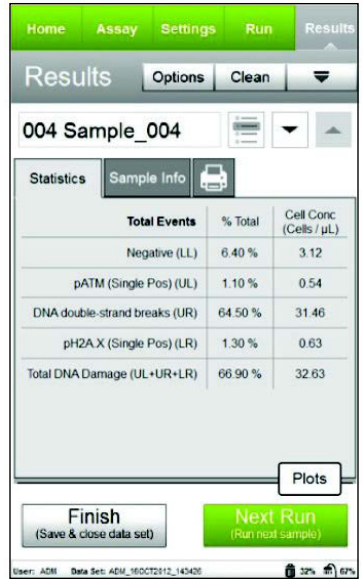
Muse® MAPK Activation Dual Detection Kit - MCH200104

Muse® System Check Kit - MCH100101

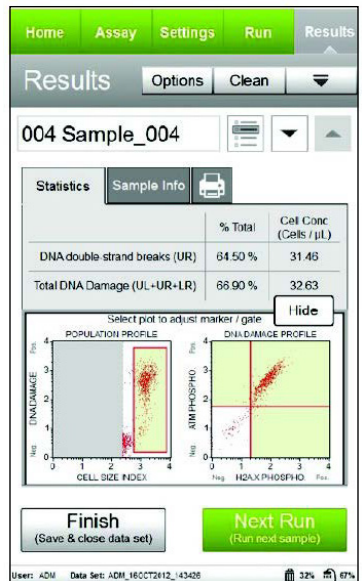
Muse® Count & Viability Kit (100T) - MCH100102

The latest version of Muse® software, which includes all assay modules, as well as the kit user's guide, can be found at: [www.luminexcorp.com/flowkits](http://www.luminexcorp.com/flowkits).

A



B



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