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Quick Reference Card

Muse® H2A.X Activation Dual Detection Kit

MCH200101

For dual detection of total H2A.X expression and phospho-specific Histone H2A.X activation in cell samples.

For Research Use Only. Not for use in diagnostic procedures.

Storage Conditions

All reagents must be stored at 2 - 8°C.

Kit Components

- 20X Anti-phospho-Histone H2A.X (Ser139), Alexa Fluor™ 555: (Part No. CS208203), 250 µL
- 20X Anti-H2A.X, PECy5: (Part No. CS208202), 300 µL
- 5X Assay Buffer: (Part No.CS202124), 55 mL
- Fixation Buffer: (Part No. CS202122), 13 mL
- 1X Permeabilization Buffer: (Part No. CS203284), 14 mL

Materials Recommended

- Guava® Muse® Cell Analyzer
- Test tubes for sample preparation and storage
- Tissue culture reagents, i.e. HBSS, PBS w/o Ca^{2+} or Mg^{2+}, cell dislodging buffers, etc.
- Micropipettes and tips
- Cells of interest in suspension
- Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Catalogue No. 16466-030, or equivalent)

Assay Protocol

Prepare cell cultures for experimentation (treated or untreated).

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Centrifuge cells at 300 x g for 5 min and wash once with 1X PBS.

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Fix cells in Fixation Buffer for 5 min on ice, followed by a washing step

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Permeabilize cells with Permeabilization Buffer for 5 min on ice, followed by a washing step

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Add 200,000 cells to each tube (treated or untreated)

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Add 10 µL of the antibody cocktail to 90 µL of 1X Assay Buffer per tube/test. Allow to incubate for 30 min at room temp (dark)

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Centrifuge cells at 300 x g for 5 minutes and wash with 1X Assay Buffer

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Resuspend in 200 µL 1X Assay Buffer

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Acquire samples on Guava® Muse® Cell Analyzer.

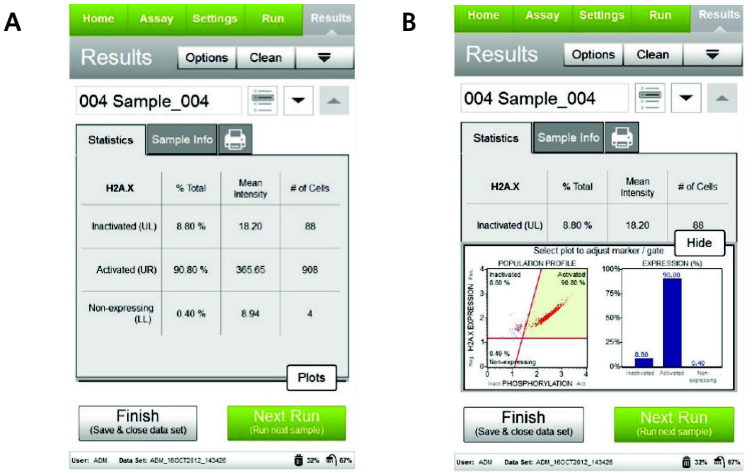
Expected Results

Healthy HeLa cells were exposed to 10 µM Etoposide for 24 hours to induce DNA damage, and then stained with the Muse® H2A.X Activation Dual Detection Kit and analyzed on the Guava® Muse Cell Analyzer. Figure A shows the results summary, while Figure B shows results displayed by both dot plot and bar graph data.

The statistics captured in this assay show the relative percentages for inactivated, activated and non-expressing (H2A.X) cells for the cell sample. Cells which express H2A.X can be seen by the data on the top two quadrants of the dot plot. Of this total cell population, 90.8% is activated upon treatment, indicating DNA damage is present, whereas 8.8% of cells remain inactivated. By presentation of both datasets, the determination of the total: phospho ratio is possible.

For more information, please refer to the comprehensive H2A.X Activation Dual Detection User's Guide, which can be found at *www.luminexcorp.com/flowkits*.

Figures A and B



Figures A and B show an example of results obtained using the Muse H2A.X Activation Dual Detection Kit

Related Products

- Muse® EGFR-RTK Activation Dual Detection Kit - MCH200102
- Muse® Bcl-2 Activation Dual Detection Kit - MCH200105
- Muse® MAPK Activation Dual Detection Kit - MCH200104
- Muse® PI3K Activation Dual Detection Kit - MCH200103
- Muse® System Check Kit - MCH100101
- Muse® Count & Viability Kit (40 mL) - 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*.

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