

Quick Reference Card

Muse® MAPK Activation Dual Detection Kit MCH200104

To measure the extent of ERK1/2 phosphorylation relative to the total ERK1/2 expression 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 Ca^{2+} or 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-ERK1/2 (Thr202/Tyr204, Thr185/Tyr187), PE: (Part No. CS208197), 250 μL
- 20X Anti-ERK1/2, PECy5: (Part No. CS208198), 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

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 5 min on ice, followed by a wash step. → Permeabilize cells with Permeabilization Buffer for 5 min on ice, followed by a wash step. → Add 200,000 cells to each tube (treated or untreated).

Add 10 μL of antibody cocktail to 90 μL 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 μL 1X Assay Buffer. → Acquire samples on Muse® Cell Analyzer



Expected Results

Results obtained using healthy Jurkat cells exposed to 100 ng/mL PMA for 5 minutes to induce a MAPK signaling cascade response, fixed, permeabilized, and then stained with the Muse® MAPK 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 each population as it is calculated within the total cell population. Cells which express ERK1/2 can be seen by the data on the top two quadrants of the dot plot (inactivated and activated), representing about 87.2% of the total cell population. But of this cell population, 75.2% is activated upon treatment, indicating activation of the MAPK signaling pathway is present. By presentation of both datasets, one can now determine the total phospho ratio within their testing samples.

For more information, refer to the comprehensive kit user's guide, which can be found at www.luminexcorp.com/flowkits. (search for Catalogue Code MCH200104)

Related Products

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Muse® EGFR-RTK Activation Dual Detection Kit - MCH200102

Muse® PI3K Activation Dual Detection Kit - MCH200103

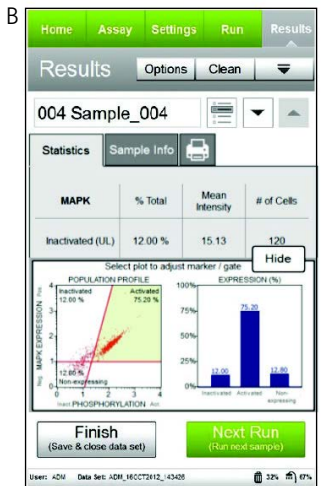
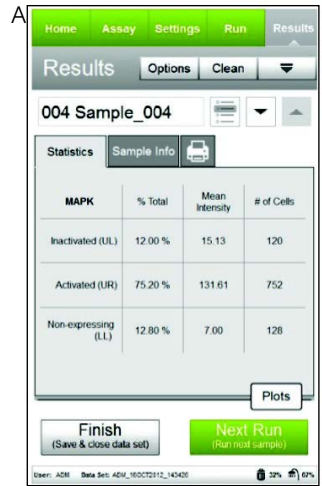
Muse® H2A.X Activation Dual Detection Kit - MCH200101

Muse® Bcl-2 Activation Dual Detection Kit - MCH200105

Muse® System Check Kit - MCH100101

Muse® Count & Viability Kit - 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.



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

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