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Quick Reference Card

Muse® Autophagy LC3 Kit (Antibody-Based)

MCH200109

To facilitate the monitoring of lipidated LC3-II in a given cell population

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-LC3 Alexa Fluor™ 555, clone 4E12: (Part No. CS208164), 250 µL
- Autophagy Detection Reagent Pack: Part No. CF200093 (stored at 2 - 8°C). Pack contains:
  - Autophagy reagent A: (Part No. CS208212), one vial (lyophilized)
  - Autophagy reagent B: (Part No. CS208215), 1 mL
  - 5X Assay Buffer: (Part No. CS202124), 55 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
- 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)

Summary of Protocol

Cell Culture and Treatment

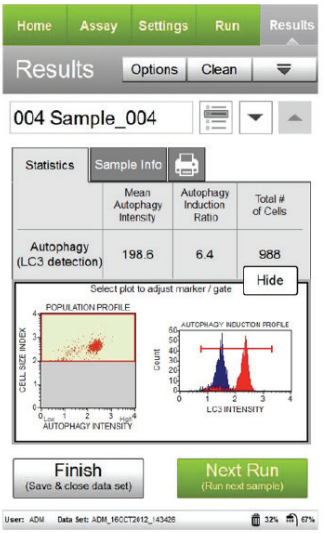
1. Culture cells at 40,000 cells per well in the 96-well plate overnight. The next day, treat with autophagy reagent A for 2-6 hours (untreated and treated).
2. Detach cells and transfer to a Muse® compatible centrifuge tube and spin at 300 x g for 5 min then remove supernatant.
3. Add 10 µL 1X autophagy reagent B per tube. Incubate for 5 min.
4. Centrifuge cells at 300 x g for 5 min and wash with 1X Assay Buffer.
5. Resuspend in 200 µL 1X Assay Buffer.
6. Acquire samples on Guava® Muse Cell Analyzer.

Expected Results

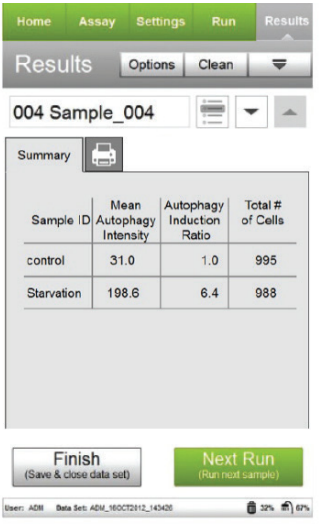
Figures A and B. HeLa cells were either starved for 4 hours to induce autophagy or kept under fed conditions, and then stained with the anti-LC3/Alexa Fluor™ 555 conjugated antibody. Samples were acquired using the Guava® Muse® Cell Analyzer and statistical results are shown here. Figure A shows the results summary for the test sample, as well as the corresponding histogram plot comparing the control versus the target sample. The statistics captured in this assay can also be illustrated by a data results summary table as shown in B. Here, the mean autophagy intensity for each sample is determined and the autophagy induction ratio is then calculated based on the ratio between the target sample (starvation) fluorescence versus the control sample. In this cell population, there is a 6.4-fold change between the control sample (e.g. no autophagy in blue) when compared to the starved sample (e.g. induced autophagy in red), indicating the presence of autophagy.

For more information, please refer to the comprehensive User's Guide for this product, which can be found at: *www.luminexcorp.com/flowkits*.

Figures A and B



A



B

Related Products

Description	Catalogue No.
Muse® Multi-Color DNA Damage Kit	MCH200107
Muse® PI3K/MAPK Dual Pathway Activation Kit	MCH200108
Muse® System Check Kit	MCH100101
Muse® Count & Viability Kit (40mL)	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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