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Measuring mitophagy via FACS with mtKeima reporter

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We use this protocol and it's working

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Abstract

Preparation of samples for measuring mitophagy levels using mtKeima reporter by fluorescence activated cell sorting (FACS).

Troubleshooting

Day 1

1 Seed cells in a 24 well plate, aiming for a confluency of ~80-90% at the time of treatment. Seed additional wells of cells not expressing any fluorescent proteins, cells expressing only mtKeima, and cells expressing only YFP-Parkin (to be used as gating controls).

Day 2

2h 3m

2 Feed all cells with standard growth media for 01:00:00 prior to treatment. 1h

3 Replace media in each well with media containing the drug you are treating with.
NOTE: Do not change the media or treat the additional wells of cells to be used for gating control.

4 01:00:00 prior to harvesting, feed the untreated wells with 0.5 mL of fresh growth media. 1h

5 At the conclusion of the treatment time point, harvest the cells using the following procedure:

5.1 Aspirate media from all wells.

5.2 Wash all wells once with 500 µL of Room temperature PBS.

5.3 Add 150 µL of trypsin to each well, and incubate cells at 37 °C for 00:01:30 1m 30s

5.4 Place plates onto ice, and harvest each sample into a separate microfuge tube on ice by resuspending each sample with 500 µL of ice cold standard growth media.

6 Centrifuge all samples at 1000 rcf for 00:01:30 at 4 °C 1m 30s

7 Carefully aspirate the supernatant from all samples.

8 Resuspend each sample in 50 µL of FACS sorting media (10% v/v FBS, 1 mM EDTA in PBS) and place into FACS analysis tubes. Keep samples on ice until immediately prior to analysis.