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# Mito-Keima assay to assess mitophagy

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ABSTRACT

This protocol details the procedure of mito-keima assay to assess mitophagy.

ATTACHMENTS

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KEYWORDS

Mito-Keima, Mitophagy, BFP-Parkin



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MATERIALS TEXT

**Growth media:**

**DMEM:**

| A | B |
|---|---|
| FBS | 10% |
| Glucose | 4.5 g/l |
| GlutaMAXTM | 1x |
| MEM NEAA | 1x |
| HEPES | 25 mM |

- 45% D-( )-Glucose **Sigma Catalog #G8769**
- GlutaMAX™ Supplement **Thermo Fisher Scientific Catalog #35050061**
- MEAA (MEM Non-Essential Amino Acids) **Gibco - Thermo Fischer Catalog #11140050**
- Antimycin A from Streptomyces sp. **Sigma – Aldrich Catalog #A8674** (made up in 100% Ethanol to 20 mg/ml)
- Oligomycin (Calbiochem, 495455; made up in DMSO to 10 mg/ml) and
- qVD **MedChemExpress Catalog #HY-12305** (made up in DMSO to 10 mM)
- 1x PBS
- **FACS buffer:** 10% FBS, [M]**0.5 millimolar (mM)** EDTA in 1xPBS

## Procedure 2h 9m

1 Seed the HeLa cells the day before the treatment day in 24 well plates.

Each well contained 0.5 ml of growth media; 120,000 cells were seeded for penta KO



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expressing BFP-Parkin, mito-Keima (mtKeima) and GFP-OPTN or -NDP52; the number of cells of other cell lines were adjusted so that the next day they are all in similar confluency with penta KO expressing BFP-Parkin, mtKeima and GFP-OPTN or -NDP52.

1.1 Control cells include unstained cells (without any fluorescence proteins), cells that only express GFP-OPTN or GFP-NDP52 (to set up compensation so that GFP signal doesn't bleach into mtKeima signal.

2 The next day, make sure the seeded cells are spreading out.

Not concentrated in the middle of the well because this can affect the results.

3 

Aspirate off the old media and treat each well with **0.5 mL** of growth media containing **4 micromolar (µM)** Antimycin A, **10 micromolar (µM)** Oligomycin and **10 micromolar (µM)** QVD for indicated times.

Make sure all drugs are vortexed well, mix the media well after adding each drug.

3.1 Treat the longest time points first.

4 

2 hours prior to harvesting, feed the untreated wells with **1 mL** of warm growth media.

5 Aftrer treatment, harvest the cells by trypsinisation.

5.1 Pre-chill eppies **On ice** .



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5.2 Aspirate the media thoroughly from the wells.

5.3 

Wash the wells with **0.5 mL** of 1x PBS.

> Make sure swirl around after adding the PBS to wash the cells properly.

5.4  7m

Aspirate 1x PBS and add **150 µL** of trypsin and incubate at **37 °C** for **00:02:00** - **00:05:00** .

> Check under microscope to make sure all the cells were trypsinised properly.

5.5 

Add **300 µL** of growth media to each well.

5.6 

Mix well with a P1000 and transfer the cells to cold eppies.

5.7  2m

Centrifuge the eppies at **1000 x g** for **00:02:00** at **4 °C** .

5.8 Aspirate off the liquid.



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6 Resuspend the cell pellets in **150 µL** of FACS buffer, transfer to pre-chilled FACS tubes and keep the samples **On ice** in the dark for analysis.



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