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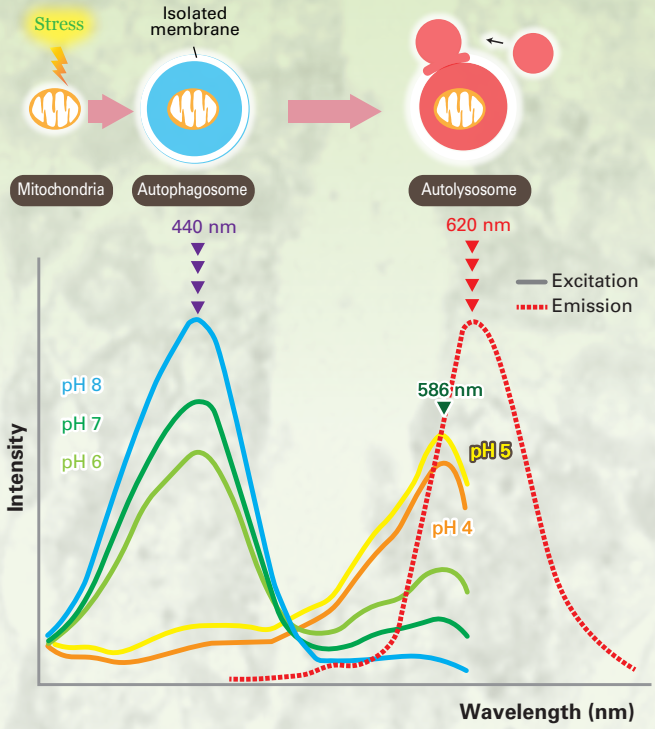
Mitophagy monitoring in living cells

- **pH dependent excitation profile**
- **Simple method for creating transfectant cells**
- **Use with any mammalian cells**

Mitophagy is the process of selective mitochondrial degradation by autophagy. It has been found to be involved in neurodegenerative and cardiovascular diseases as well as cancer. Keima-Red can detect mitophagy in living cells by using a unique excitation profile and a mitochondria targeting tag.

Principle for mitophagy detection

Keima-Red has a unique pH dependent excitation profile. Keima-Red is excited at 440 nm under neutral pH (> 6) and 586 nm under acidic pH (< 4). The emission profile is constant under any pH environment. Under normal conditions, mitochondria are surrounded by neutral pH. Once mitochondria are damaged, mitophagy degrades them under acidic conditions. Mitochondria targeted Keima-Red allows monitoring of mitophagy in living cells by the change of of environmental pH around mitochondria using its excitation profile.



Katayama, H. et al., Chemistry & Biology 18, 1042-1052 (2011)

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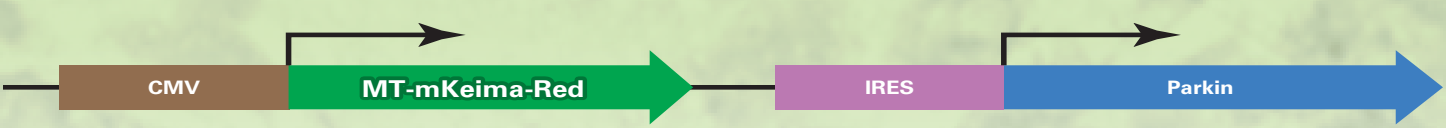
pMitophagy Keima-Red-Parkin

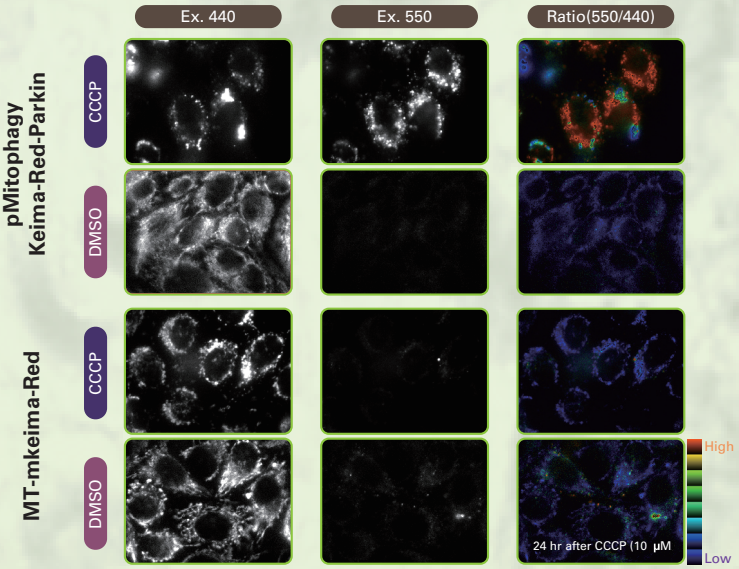


Parkin is a fundamental component in mitophagy cascade. Until now, mitophagy was monitored using individual transfected expression vectors Keima-Red and Parkin. MBL has designed a new plasmid to simplify the transfection process. pMitophagy Keima-Red-Park2 is designed to establish transfectants easily using one expression vector that consists of an IRES-based bicistronic expression system. This vector is compatible with high content analysis (HCA) using IN Cell Analyzer.

Ratio Imaging

Mitophagy flux is analyzed by the fluorescent intensity ratio of 440 nm and 550 nm. A strong signal at 440 nm indicates that mitophagy did not occur while a strong signal at 550 nm indicates mitophagy occurred.

CCCP: Mitophagy inducer
Cell: HeLa (Parkin negative cell)

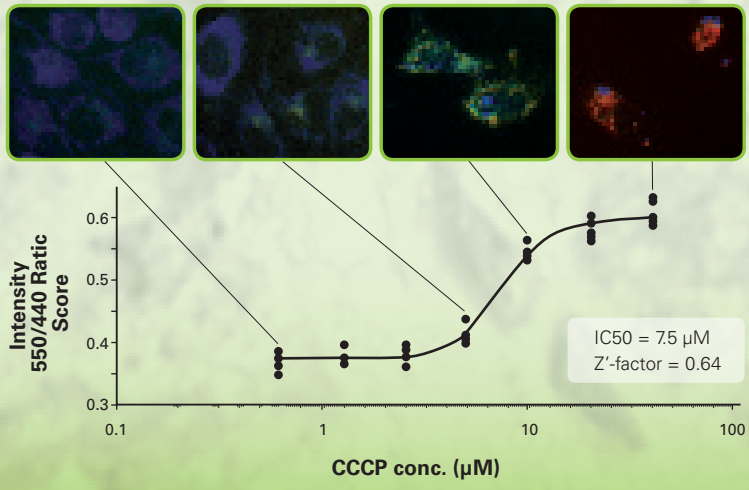


High content analysis by IN Cell Analyzer

Mitophagy was detected using IN Cell Analyzer 1000. We obtained reasonable IC50 value and a sufficient Z'-factor. pMitophagy Keima-Red -Parkin provides a robust assay for mitophagy monitoring in living cells using high content analyzer.

Zhang, JH. et al., J Biomol Screen 4, 67-73 (1994)

Code no.	Product
AM-V0259M	pMitophagy Keima-Red-Parkin (Kan)
AM-V0259HM	pMitophagy Keima-Red-Parkin (Hyg)



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