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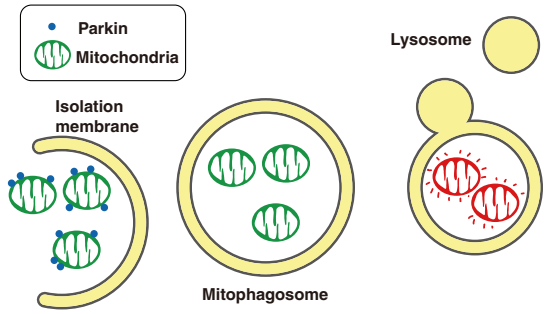
Detection of mitophagy with Keima-Red

What is mitophagy?

Mitophagy is a type of autophagy that selectively degrades mitochondria, and is involved in the turnover of damaged mitochondria. This process is thought to defend the body from diseases resulting from mitochondrial dysfunction. The Parkinson's disease gene product, Parkin (ubiquitin ligase), plays a critical role in the induction of mitophagy. Parkin is recruited to the outer membrane of damaged and depolarized mitochondria. Ubiquitin is subsequently added to the outer membrane of damaged mitochondria by the ubiquitin ligase activity of Parkin. Mitophagy is induced through the recognition of the ubiquitin modification.



Plasmid vector for monitoring mitophagy activity, pMitophagy Keima-Red mPark2

This vector is designed for labeling mitochondria with the fluorescent protein mKeima-Red (monomeric with an emission maximum at 620 nm). mKeima-Red is tagged with a mitochondrial localization signal, and is co-expressed in the cells with Parkin (ubiquitin ligase), which plays a critical role in the induction of mitophagy. Mitophagy can be detected and visualized due to the changes in the excitation spectrum of mKeima-Red before and after induction of mitophagy by drug treatment.

Features

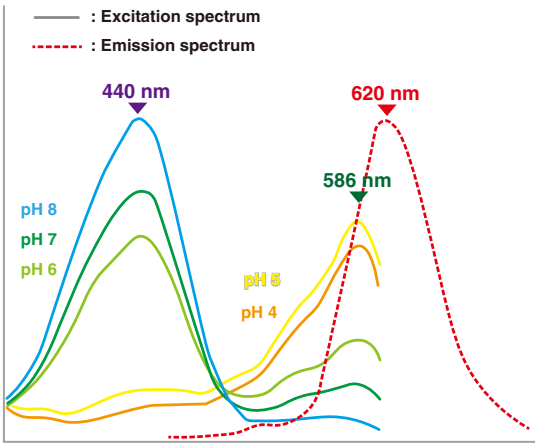
◎ **mKeima-Red (tagged with a mitochondrial localization signal) and Parkin are co-expressed from a single construct.**

*MT-mKeima-Red expression vectors without Parkin are also available.

Features of Keima-Red: pH biosensor

mKeima-Red is a fluorescent protein with an emission maximum at 620 nm. The excitation spectrum changes depending on the pH of the environment.

Excitation and emission spectra of mKeima-Red



* There is no change in the emission spectrum.

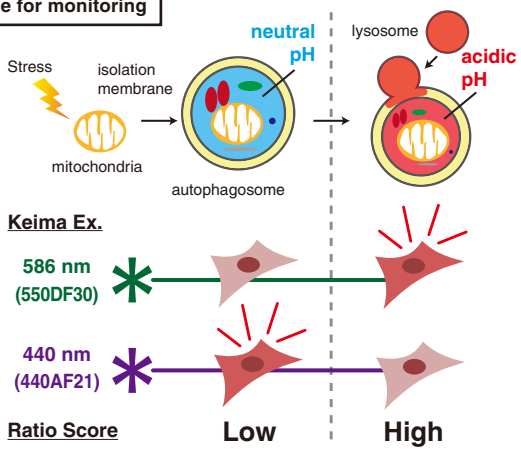
Changes in the excitation spectrum

Neutral (>pH 6)	Acidic (<pH 5)
440 nm	586 nm

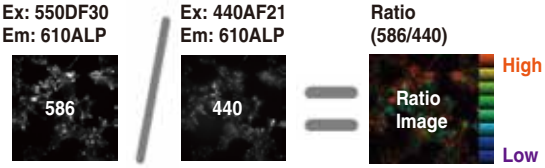
The principle for monitoring and the analysis method

Images are taken with excitation wavelengths of 440 and 586 nm (the excitation maxima in a neutral and acidic environment, respectively) and processed for ratio imaging (586 nm/440 nm). The high ratio is shown in red, and the low ratio is shown in blue. Keima has a low ratio score (colored in blue) in a neutral environment and has a high ratio score (colored in red) in an acidic environment. The change from blue to red indicates the induction of mitophagy.

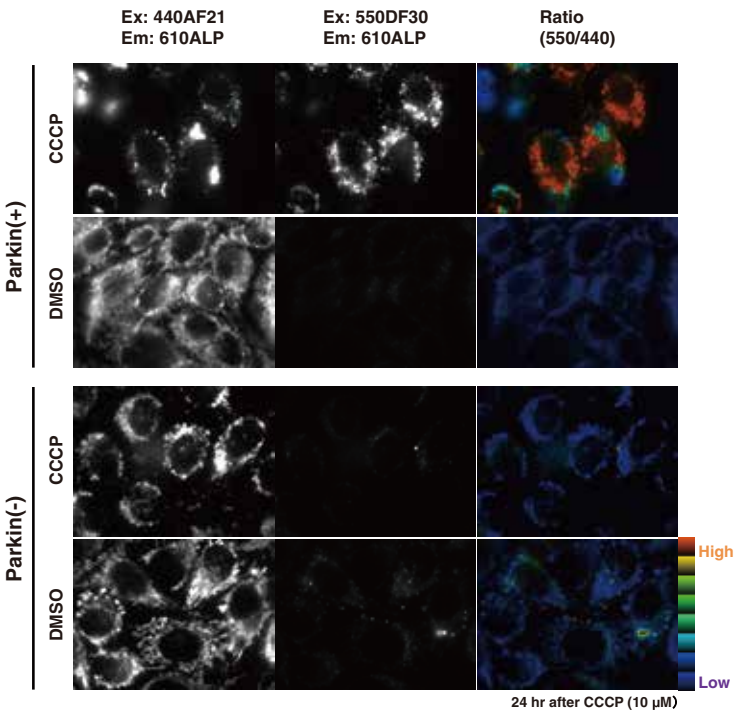
Principle for monitoring



Analysis method

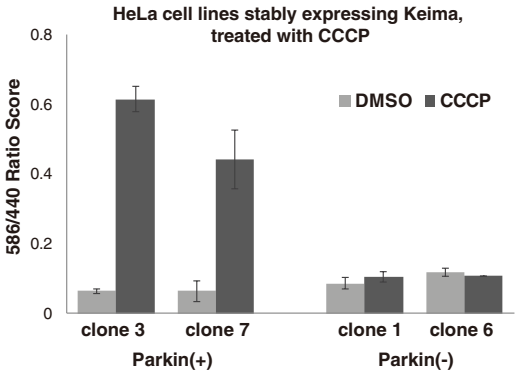


Ratio imaging



The Ratio (586/440) panels show the ratio of fluorescence intensities observed with excitation filters 550DF30 and 440AF21. A higher ratio indicates greater activation of mitophagy.

Quantitative analysis



CCCP : Mitochondrial membrane depolarizer
DMSO: Control

■ Stable cell lines (HeLa cells)

Parkin(+): Transfected with MT-mKeima-Red-IRES-Park2
Parkin(-): Transfected with MT-mKeima-Red

■ Assay method

Cells were imaged 24 hours after treatment with CCCP (10 μM) or DMSO.

■ Filter settings

440 nm (Ex: 440AF21, Em: 610ALP, DM: 590DRLP)
586 nm (Ex: 550DF30, Em: 610ALP, DM: 590DRLP)

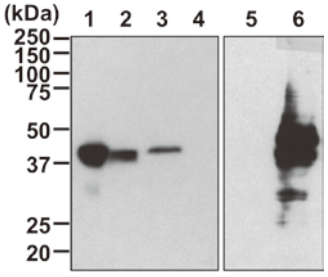
Product list

Code No.	Product name	Size
AM-V0259M	pMitophagy Keima-Red mPark2 (Kan)	20 μg
AM-V0259HM	pMitophagy Keima-Red mPark2 (Hyg)	20 μg
AM-V0251M	*CoralHue*® Mitochondria-targeted mKeima-Red (pMT-mKeima-Red)	20 μg
AM-V0251HM	*CoralHue*® Mitochondria-targeted monomeric Keima-Red (Hyg)	20 μg

Anti-Parkin mAb

Code No.	Product name	Clone	Isotype	Size	Application	Species cross-reactivity
M230-3	Anti-Parkin mAb	Par6	Mouse IgG2aκ	100 μg/100 μL	WB	Human, Mouse, Rat

■ Western blotting



Lane 1: Rat brain lysate, 20 μg
Lane 2: Mouse brain lysate, 20 μg
Lane 3: PC12
Lane 4: HeLa
Lane 5: HEK293T
Lane 6: Human Parkin/ HEK293T

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