

**Fluorescent Protein Expression Plasmid**  
**pMitophagy Keima-Red mPark2 (Kan)**

Code No.  
AM-V0259M

Quantity  
20 µg

**VECTOR DESCRIPTION:**

Mitophagy detection vectors are designed for the co-expression of *MT-mKeima-Red* (**CoralHue™** Mitochondria-targeted monomeric Keima-Red) gene and mouse *Parkin* (*mPark2*) gene from the internal ribosome entry site (IRES), as parts of a bicistronic transcript in mammalian cells. *Keima-Red* has been cloned from *Montipora* sp., the stony coral in Kerama islands located at the southwest of Japan. A monomeric version of **CoralHue™** Keima-Red (mKeima-Red) displays a bimodal excitation spectrum with peaks at 440 and 586 nm in neutral and acidic solutions, respectively, and an emission maximum at 620 nm. Mitochondrial targeting of mKeima-Red is achieved by introducing a mitochondrial signal sequence at the *N*-terminus of mKeima-Red.

MT-mKeima-Red has been used to monitor mitophagy activity, a selective degradation of mitochondria by autophagy while Park2 is essential for the induction of mitophagy. It is known that some cell lines such as HeLa and HEK293 commonly used for mitophagy studies show little or no expression of endogenous *Parkin*. Thus, expression of exogenous *Parkin* on the vectors could help monitoring of mitophagy activity.

**SOURCE:**

The **CoralHue™** Keima-Red gene was originally cloned from the stony coral “Komon-Sango (*Montipora* sp.)”

**FORMULATION:**

Dry form. Reconstitute with distilled water or TE before use.

**PURITY:**

A260/A280 > 1.5

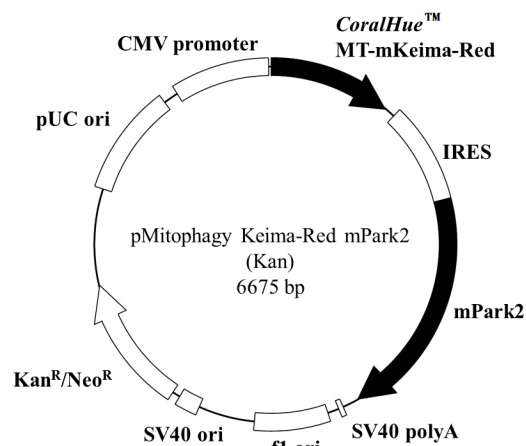
**STORAGE:**

Store at -20°C

**SEQUENCE LANDMARKS (bases):**

**CoralHue™** MT-mKeima-Red (Including Stop Codon):  
bases 1-750  
IRES: 815-1396  
mPark2: 1400-2794  
SV40 polyA: bases 2890-2924  
fl origin: bases 2987-3442  
SV40 origin: bases 3783-3918  
Kanamycin/Neomycin resistance gene: bases 3966-4760  
pUC origin: bases 5345-5988  
CMV promoter: bases 6081-6656

**VECTOR MAP:**



**NOTE:**

AM-V0259M pMitophagy Keima-Red mPark2 (Kan) contains the kanamycin/neomycin resistance gene. It allows selection of stable transformants with kanamycin, neomycin or G418 in *Escherichia coli* and G418 in mammalian cells. It is recommended to determine the optimal concentration of antibiotics for the cells in use.

**GenBank:**

Accession Numbers: AB209969 (*mKeima-Red*), AB019558 (*mPark2*).

**REFERENCES:**

- 1) Sun, N. *et al.*, *Molecular Cell* **60**, 685–696 (2015)
- 2) Vinay Choubey, M. C. *Autophagy* **10**, 1105–1119 (2014)
- 3) Bingol, B., *et al.*, *Nature* **510**, 370–375 (2014)
- 4) Narendra, D. P., *et al.*, *Hum. Mol. Genet.* ddt106 (2013)
- 5) Togashi, K. *et al.*, *PLoS ONE* **8**, e81313 (2013)
- 6) Safiulina, D. & Kaasik, A. *PLoS Biol* **11**, e1001755 (2013)
- 7) Katayama, H., *et al.*, *Chemistry & Biology* **18**, 1042–1052 (2011)
- 8) Kogure, T., *et al.*, *Nat. Biotechnol.* **24**, 577-581 (2006)

**Related Products:**

- AM-V0251M **CoralHue™** Mitochondria-targeted monomeric Keima-Red (Kan)
- AM-V0251HM **CoralHue™** Mitochondria-targeted monomeric Keima-Red (Hyg)
- AM-V0259HM pMitophagy Keima-Red (Hyg)

**INTENDED USE:**

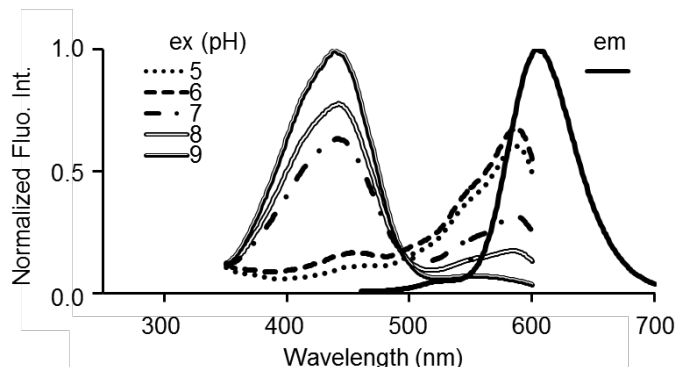
For Research Use Only. Not for use in diagnostic procedures.

**CoralHue™ mKeima-Red: 222 amino acids (without MT signal sequence)**

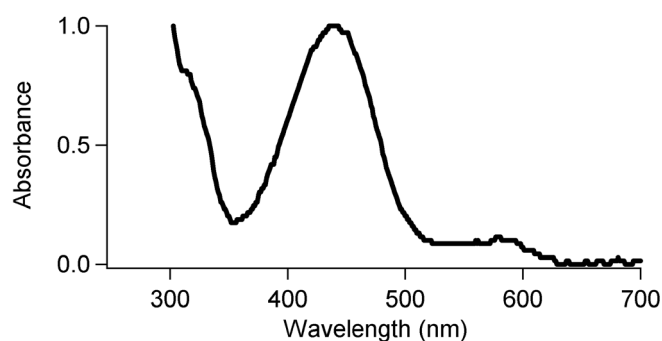
	*Excit./Emiss.Maxima (nm)	*Extinction Coefficient( $M^{-1}cm^{-1}$ )	*Fluorescence Quantum Yield	pH sensitivity
mKeima-Red	440/620	14,000 (440 nm)	0.24	$pK_a=6.5$

\*These properties were measured in pH 7.4.

**Excitation and Emission Spectrum:**



**Absorption Spectrum:**



**Recommended Filters:**

- Excitation filters
  - 440AF21 (Omega Optical) for peak at 440 nm
  - 550DF30 (Omega Optical) for peak at 586 nm
- Dichroic mirror
  - 590DRLP (Omega Optical)
- Emission filter
  - 610ALP (Omega Optical)

**CoralHue™ MT-mKeima-Red**

**1) DNA sequence**

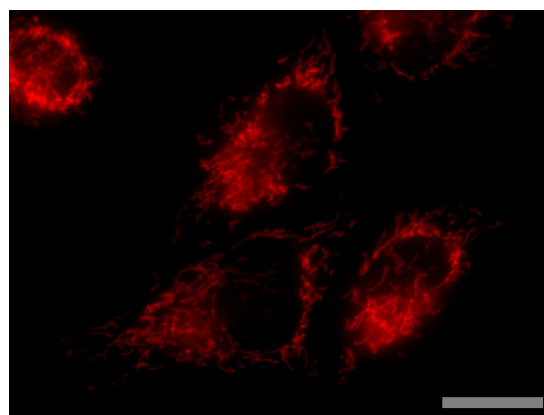
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 CACTACTTTGAGGTGGAAGGCATGAAAAGGAAAGCCTTACGAGGGA  
 GAGCAGACAGTAAAGCTCACTGTCACCAAGGGTGGACCTCTGCCATT  
 GCTTGGGATATTTATCACCACAGCTTCAAGTACGGAAGCATACCATT  
 ACCAAGTACCCTGAAGACATCCCTGATTATTTCAAGCAGTATTCCCT  
 GAGGGATATACATGGGAGAGGAGCATGAACCTTGAAGATGGTGCAAT  
 TGTACTGTCAGCAATGATCCAGCATCCAAGGCAACTGTTTCATCTAC  
 AATGTCAAAATCTCTGGTGAGAACTTTCCCTCCAATGGACCTGTTATG  
 CAGAAGAAGACACAGGGCTGGGAACCCAGCACTGAGCGTCTCTTTGCA  
 CGAGATGGAATGCTGATAGGAAACGATTATATGGCTCTGAAGTTGGAA  
 GGAGTGGTCACTATTTGTGTGAATTTAAATCTACTTACAAGGCAAAG  
 AAGCTGTGAGGATGCCAGGGCCACGAGATTGACCGCAAAGTGGAT  
 GTAACCAAGTACAACAGGGATTACACATCTGTTGAGCAGTGTGAAATA  
 GCCATTGCACGCCACTCTTTGCTCGGT

(Underlined sequences in red are from cytochrome C oxidase subunit IV.)

**2) Amino acid sequence**

MLSLRQSIIRFFKPATRTLCSRRAAAGTMVSVIAKQMTYKVYMSGTVNG  
 HYFEVEGDGKGPYEGEQTVKLTVTKGGPLPFAWDILSPQLQYGSIPF  
 TKYPEDIPDYFKQSFPEGYTWERSMNFEDGAVCTVSNDSIQGNCFIY  
 NVKISGENFPPNGPVMQKKTQGWEPSTERLFARDGMLIGNDYMALKLE  
 GGGHYLCEFKSTYKAKKPVMPGRHEIDRKLDTVSHNRDYSVEQCEI  
 AIARHSLLG

(Underlined sequences in red are from cytochrome C oxidase subunit IV.)



**CoralHue™ MT-mKeima-Red expression in HeLa cells.**  
bar: 20  $\mu m$

**CoralHue™ Keima-Red** is a product of co-development with Dr. Atsushi Miyawaki at the Laboratory for Cell Function and Dynamics, the Brain Science Institute, and the Institute of Physical and Chemical Research (RIKEN).

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