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

Amalgaam MBL

Fluorescent Protein Expression Plasmid

pMitophagy Keima-Red mPark2 (Hyg)

Code No.	Quantity
AM-V0259HM	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 *Montipara* 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 *Park2*. Thus, expression of exogenous *Park2* 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

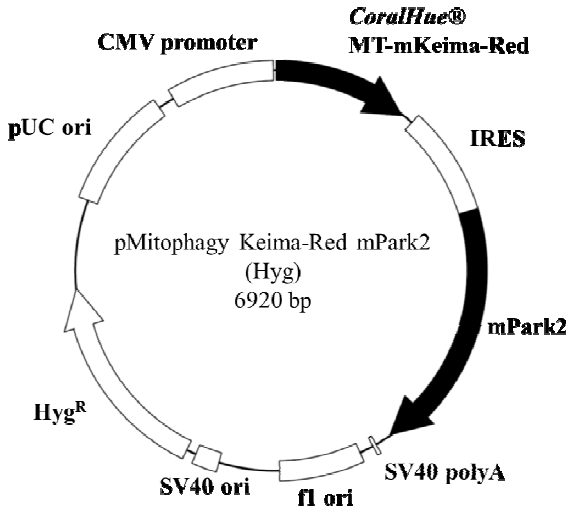
STORAGE:

Store at -20°C

SEQUENCE LANDMARKS (bases):

CoralHue® MT-mKeima-Red (Including Stop Codon): 1-750
IRES: 815-1396
mPark2: 1400-2794
SV40 polyA: 2890-2924
f1 origin: 2987-3442
SV40 origin: 3783-3918
Hygromycin B resistance gene: 3969-5090
pUC origin: 5590-6233
CMV promoter: 6326-6901

VECTOR MAP:



NOTE:

AM-V0259HM pMitophagy Keima-Red mPark2 (Hyg) contains the hygromycin B resistance gene. It allows selection of stable transformants of *Escherichia coli* and mammalian cells with hygromycin B. It is recommended to determine the optimal concentration of hygromycin B for the cells in use.

GenBank:

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

REFERENCES:

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

INTENDED USE:

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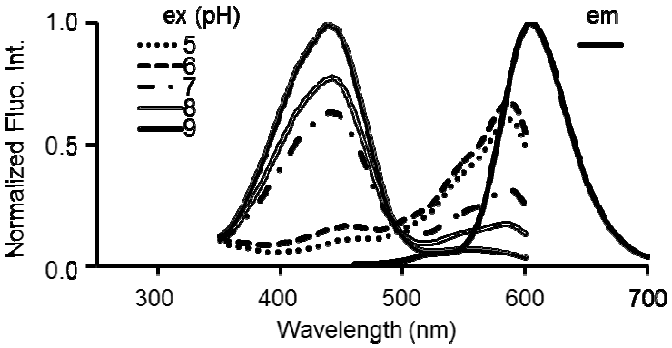
CoralHue® mKeima-Red: 222 amino acids (without MT signal sequence)

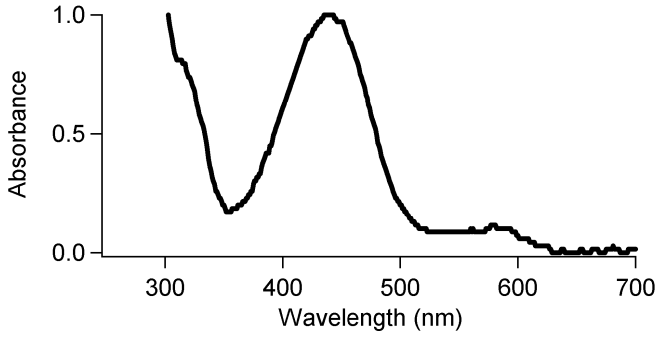
	*Excit./Emiss.Maxima (nm)	*Extinction Coefficient($M^{-1}cm^{-1}$)	*Fluorescence Quantum Yield	pH sensitivity
mKeima-Red	440/620	14,000 (440 nm)	0.24	p*K* a=6.5

*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

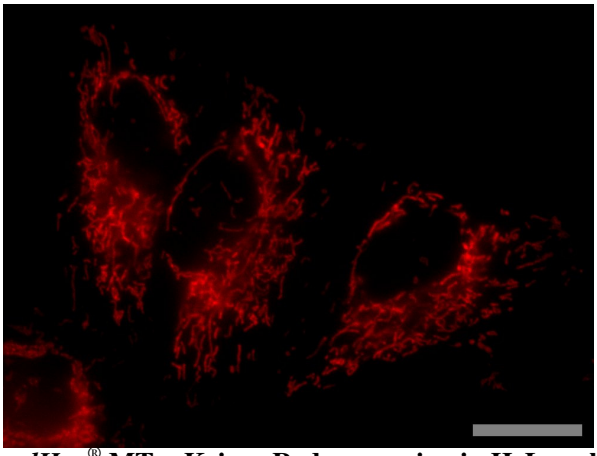
ATGCTGAGCCTGCGCCAGAGTATCCGCTTCTTCAAGCCCGCCACCAGG
ACTCTGTGCAGTTCCAGGGCGGCCGCGGGGACAATGGTGAGTGTGATC
GCTAAACAAATGACCTACAAGGTTTATATGTCAGGCACGGTCAATGGA
CACTACTTTGAGGTCGAAGGCGATGGAAAAGGAAAGCCTTACGAGGGA
GAGCAGACAGTAAAGCTCACTGTCACCAAGGGTGGACCTCTGCCATTT
GCTTGGGATATTTTATCACCACAGCTTCAGTACGGAAGCATACCATTC
ACCAAGTACCCTGAAGACATCCCTGATTATTTCAAGCAGTCATTCCCT
GAGGGATATACATGGGAGAGGAGCATGAACTTTGAAGATGGTGCAGTG
TGTACTGTCAGCAATGATTCCAGCATCCAAGGCAACTGTTTCATCTAC
AATGTCAAAATCTCTGGTGAGAACTTTCCTCCCAATGGACCTGTTATG
CAGAAGAAGACACAGGGCTGGGAACCCAGCACTGAGCGTCTCTTTGCA
CGAGATGGAATGCTGATAGGAAACGATTATATGGCTCTGAAGTTGGAA
GGAGGTGGTCACTATTTGTGTGAATTTAAATCTACTTACAAGGCAAAG
AAGCCTGTGAGGATGCCAGGGCGCCACGAGATTGACCGCAAACTGGAT
GTAACCAGTCACAACAGGGATTACACATCTGTTGAGCAGTGTGAAATA
GCCATTGCACGCCACTCTTTGCTCGGT

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

2) Amino acid sequence

MLSLRQSIRFFKPATRTLCSSRAAAGTMVSVIAKQMTYKVYMSGTVNG
HYFEVEGDGKGKPYEGEQTVKLTVTKGGPLPFAWDILSPQLQYGSIPF
TKYPEDIPDYFKQSFPEGYTWERSMNFEDGAVCTVSNDSSIQGNCFIY
NVKISGENFPPNGPVMQKKTQGWEPSTERLFARDGMLIGNDYMALKLE
GGGHYLCEFKSTYKAKKPVRMPGRHEIDRKLDVTSHNRDYTSVEQCEI
AIARHSLLG

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



***CoralHue*® MT-mKeima-Red expression in HeLa cells.**
bar: 20 µm

Related Products:

AM-V0251M	***CoralHue*®** Mitochondria-targeted monomeric Keima-Red (Kan)
AM-V0251HM	***CoralHue*®** Mitochondria-targeted monomeric Keima-Red (Hyg)
AM-V0259M	pMitophagy Keima-Red (Kan)