

Fucci (*Fluorescent Ubiquitination-based Cell Cycle Indicator*) series  
**pFucci-G<sub>1</sub> Orange (Expression vector)**

Code No.	Quantity
AM-V9003M	20 µg

**VECTOR DESCRIPTION:**

AM-V9003M pFucci-G<sub>1</sub> Orange is a mammalian expression vector encoding *CoralHue*<sup>®</sup> monomeric Kusabira-Orange 2 (mKO2) fused to a part of human Cdt1 (hCdt1: Cdc10 dependent transcript 1). "Fucci" stands for *Fluorescent Ubiquitination-based Cell Cycle Indicator*.

Cdt1 is the DNA replication licensing factor. It accumulates during G<sub>1</sub> phase in nuclei, but is degraded during S/G<sub>2</sub>/M phases by ubiquitin-mediated proteolysis. A part of hCdt1 (30-120) is also degradable in a cell cycle dependent manner.

*CoralHue*<sup>®</sup> mKO2 has been generated from dimeric *CoralHue*<sup>®</sup> Kusabira-Orange (KO). *CoralHue*<sup>®</sup> mKO2 has a feature of rapid maturation.

**SOURCE:** The *CoralHue*<sup>®</sup> KO gene was cloned from a stony coral (*Fungia concinna*).

**FORMULATION:** Dry form. Reconstitute with distilled water or TE before use.

**PURITY:** A260/A280 > 1.5

**STORAGE:** Stored at -20°C

**SEQUENCE LANDMARKS:**

Fucci-G<sub>1</sub> Orange: bases 64-1041  
CMV promoter: bases 4394-4956  
SV40 polyA: bases 1204-1338  
Kanamycin/Neomycin resistance gene: bases 2281-3072  
pUC origin: bases 3663-4303  
f1 origin: bases 1301-1756  
SV40 origin: bases 2097-2232

**REFERENCES:**

- 1) Sakaue-Sawano, A., *et al.*, *Cell*. **132**, 487-498 (2008)
- 2) Nakayama, K. I., *et al.*, *Nat. Rev. Cancer*. **6**, 369-381 (2006)
- 3) Blow, J. J., and Dutta, A., *Nat. Rev. Mol. Cell Biol.* **6**, 476-486 (2005)
- 4) Nishitani, H., *et al.*, *J. Biol. Chem.* **279**, 30807-30816 (2004)
- 5) Karasawa, S., *et al.*, *J. Biol. Chem.* **278**, 34167-71 (2003)
- 6) Nishitani, H., *et al.*, *Nature*. **404**, 625-628 (2000)

**INTENDED USE:**

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

**GenBank:**

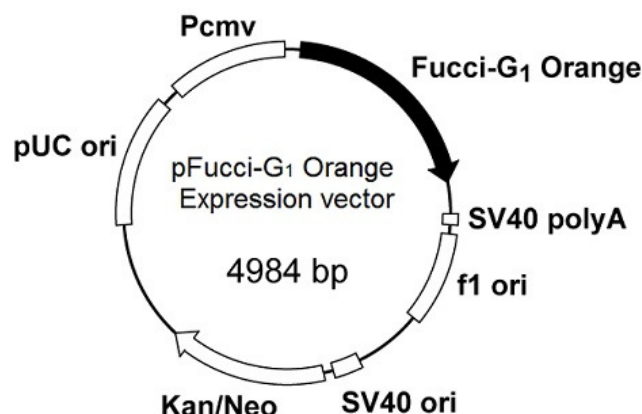
Accession Numbers: AB370332

**NOTICES:**

- 1) Val (encoded by GTG) is inserted as the second amino acid of *CoralHue*<sup>®</sup> mKO2 to form the Kozak sequence.
- 2) It is recommended that Fucci be stably expressed.
- 3) This vector contains the neomycin resistance gene to allow selection of stable transformants using G418. To successfully generate a stable cell line, you need to determine the minimum concentration of G418 required to kill your untransfected host cells.

**RELATED PRODUCTS:**

AM-V9016M pFucci-S/G<sub>2</sub>/M Green (Expression vector)  
AM-V9010M pFucci-S/G<sub>2</sub>/M Green-Hyg (Expression vector)  
AM-V9001M pFucci-G<sub>1</sub> Orange (Cloning vector)  
AM-V9014M pFucci-S/G<sub>2</sub>/M Green (Cloning vector)  
AM-V9030M pFucci-S/G<sub>2</sub>/M Green (N+C)-Hyg (Expression vector)  
AM-V9034M pFucci-S/G<sub>2</sub>/M Green (N+C) (Cloning vector)



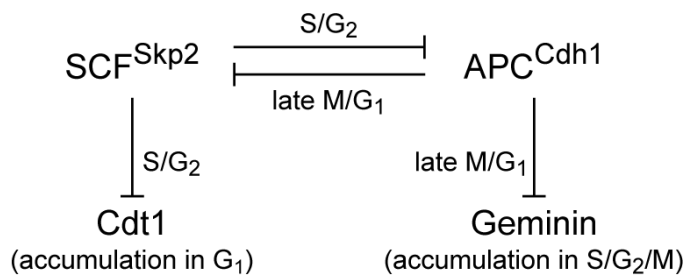


Fig 1. Cell cycle regulation by SCF<sup>Skp2</sup> and APC<sup>Cdh1</sup>.

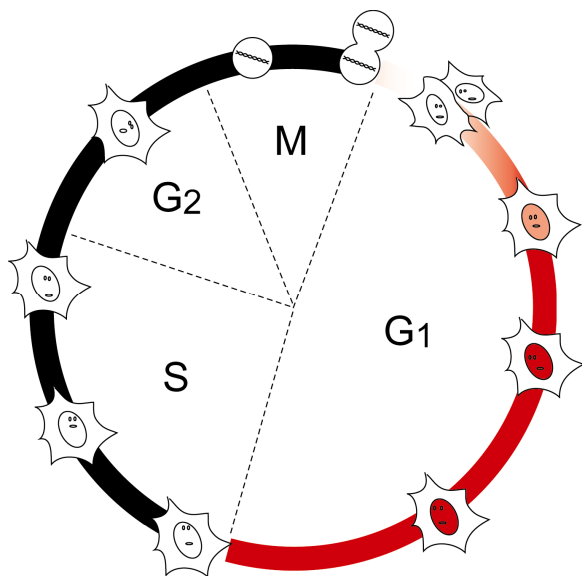


Fig 2. Schematic of the cell cycle specific fluorescence of Fucci-G<sub>1</sub> Orange.

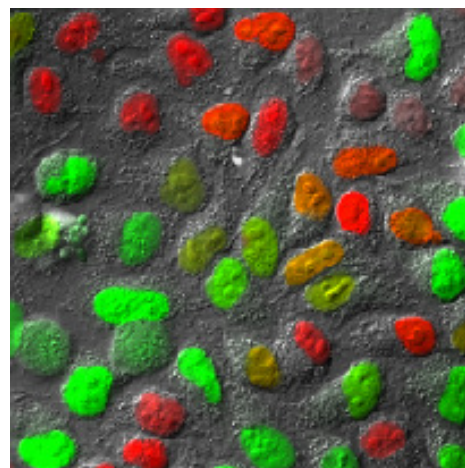
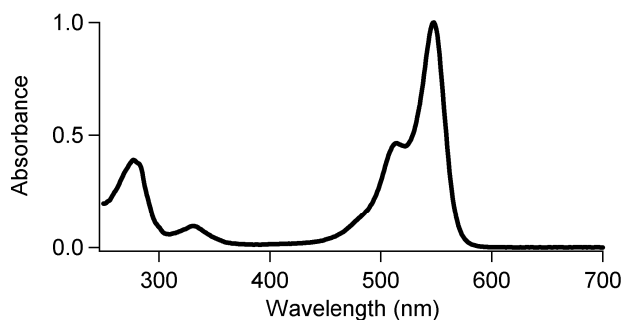
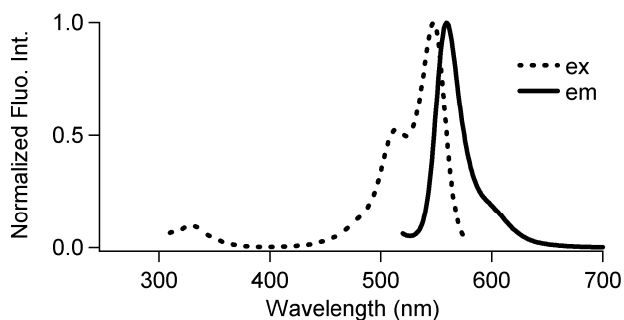


Fig 3. HeLa cells stably expressing Fucci-G<sub>1</sub> Orange and Fucci-S/G<sub>2</sub>/M Green. Fucci effectively labels individual nuclei in G<sub>1</sub> phase orange and those in S/G<sub>2</sub>/M phases green.

**CoralHue<sup>®</sup> mKO2:** 218 amino acids

	Excit./Emiss.Maxima (nm)	Extinction Coefficient(M <sup>-1</sup> cm <sup>-1</sup> )	Fluorescence Quantum Yield	pH sensitivity
mKO2	551/565	63,800 (551 nm)	0.62	pK <sub>a</sub> =5.5



## Fucci-G<sub>1</sub> Orange

### 1) DNA sequence

ATGGTGAGTGTGATTAACCAGAGATGAAGATGAGGTA CTACAT  
GGACGGCTCCGTCAATGGGCATGAGTTCACAATTGAAGGTGAAG  
GCACAGGCAGACCTTACGAGGGACATCAAGAGATGACACTACGC  
GTCACAATGGCCGAGGGCGGGCCAATGCCTTTGGCGTTTGA CT  
AGTGTACACAGTGTCTGTACGGCCACAGAGTATTTACTAAAT  
ATCCAGAAGAGATACCAGACTATTTCAAACAAGCATTTCTGAA  
GGCCTGTGATGGGAAAGGTGTTGGAGTTCGAAGATGGTGGGTC  
CGCTTCACTCAGTGCATATAAGCCTTAGAGGAAACACCTTCT  
ACCACAAATCCAAATTTACTGGGGTTAACTTTCTGCGGATGGT  
CCTATCATGCAAAACCAAAGTGTGATTGGGAGCCATCAACCGA  
GAAAATTACTGCCAGCGACGGAGTTCTGAAGGGTGATGTTACGA  
TGTACCTAAAAGTTGAAGGAGGCGGCAATCACAAATGCCAAATG  
AAGACTACTTACAAGGCGGCAAAGAGATTCTTGAATGCCAGG  
AGACCATTACATCGGCCATCGCCTCGTCAGGAAAACCGAAGGCA  
ACATTACTGAGCAGGTAGAAGATGCAGTAGCTCATTACTCAATG  
TTGCCTTCCAGGGCGGATATCCATCACACTGGCGCCGCTCGA  
GCCAGCCCCGCCAGGCCCGCACTCCGCGCCCCGGCCTCCGCTA  
CCAGTGGCAGCCGCAAGCGCCCGCCCGCCCGCCCGCCCGCGGA  
CGCGACCAGGCCAGGCCACCGCCCGCAGGAGACTGCGGCTGTC  
GGTGGAGGAGGTTTCCAGGCCAGTACCCCGAGGCCCGAGACA  
TCCAGCCTGCCCTTCTCCGGGCCAGAAGATAAAGAAATCCACC  
CCGGCAGCAGGTACGCCGCCACCTGACATCCGCGCAGGACCA  
GGACACCATC

### 2) Amino acid sequence

MVSVIKPEMKMRYMDG SVNGHEFTIEGEGTGRPYEGHQEMTLR  
VTMAEGGPMPFAFDLVSHVFCYGHRVFTKYPEEIPDYFKQAFPE  
GLSWERSLEFEDGGSASVSAHISLRGNTFYHKSFTGVNFPADG  
PIMQNGSVDWEPSTEKITASDGLKGDVTMYLKLEGGNHNKQCM  
KTTYKAAKEILEMPGDHYIGHRLVRKTEGNITEQVEDAVAHYSM  
LPSQGGYP SHWRPLESPARPALRAPASATSGSRKRARPPAAPG  
RDQARPPARRRLRLSVDEVSSPSTPEAPDIPACPSPGQKIKKST  
PAAGQPPHLTSAQDQDTI

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**CoralHue<sup>®</sup> mKO** 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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Patent Nos. JP4258724, US7226993 and EP1700913 and patents pending.