

Fucci (*Fluorescent Ubiquitination-based Cell Cycle Indicator*) series
pFucci-G₁ Orange (Expression vector)

Code No.	Quantity
AM-V9003M	20 µg

VECTOR DESCRIPTION:

AM-V9003M pFucci-G₁ Orange is a mammalian expression vector encoding *CoralHue*[®] 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 G₁ phase in nuclei, but is degraded during S/G₂/M phases by ubiquitin-mediated proteolysis. A part of hCdt1 (30-120) is also degradable in a cell cycle dependent manner.

CoralHue[®] mKO2 has been generated from dimeric *CoralHue*[®] Kusabira-Orange (KO). *CoralHue*[®] mKO2 has a feature of rapid maturation.

SOURCE: The *CoralHue*[®] 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-G₁ 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**, 34167-34171 (2003)
- 6) Nishitani, H., *et al.*, *Nature*. **404**, 625-628 (2000)

INTENDED USE:

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

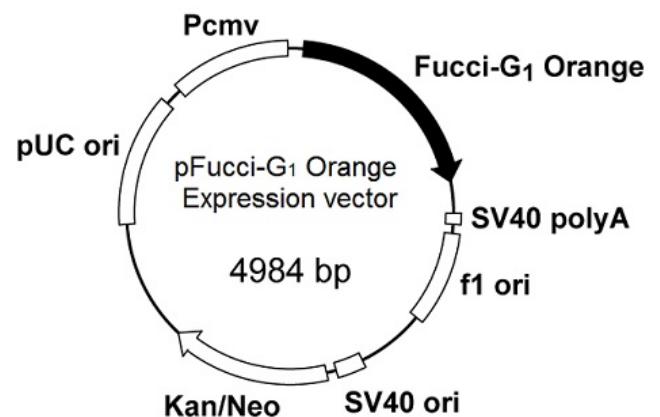
GenBank:

Accession Numbers: AB370332

NOTICES:

- 1) Val (encoded by GTG) is inserted as the second amino acid of *CoralHue*[®] 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.

For more information, please visit our web site.
<https://ruo.mbl.co.jp/>



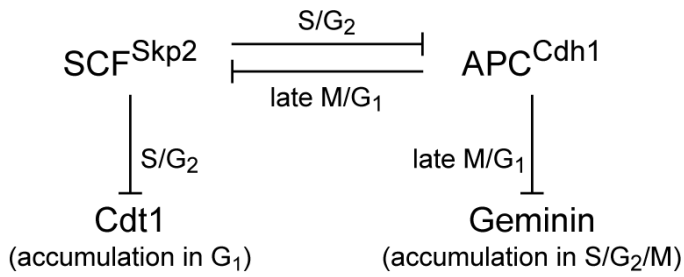


Fig 1. Cell cycle regulation by SCF^{Skp2} and APC^{Cdh1}.

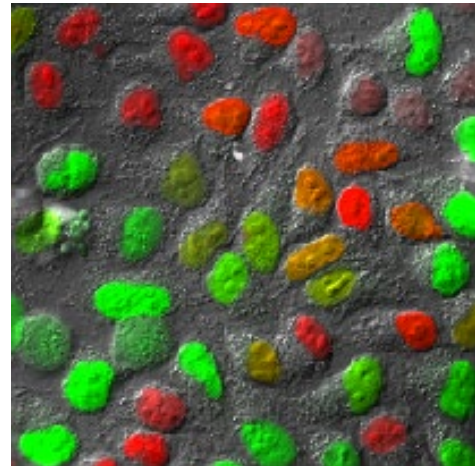


Fig 3. HeLa cells stably expressing Fucci-G₁ Orange and Fucci-S/G₂/M Green. Fucci effectively labels individual nuclei in G₁ phase orange and those in S/G₂/M phases green.

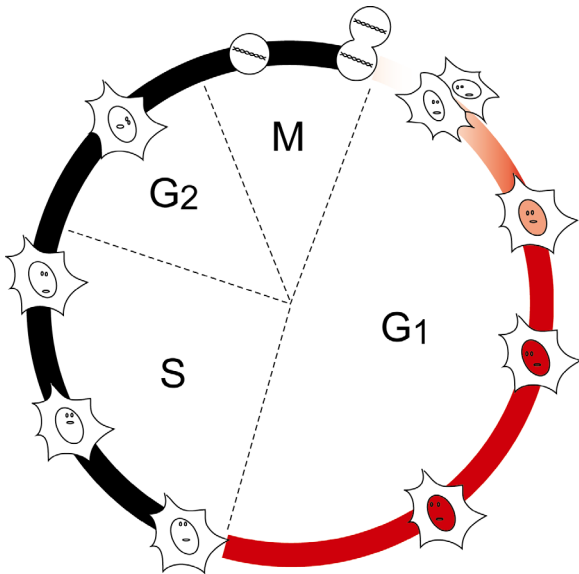
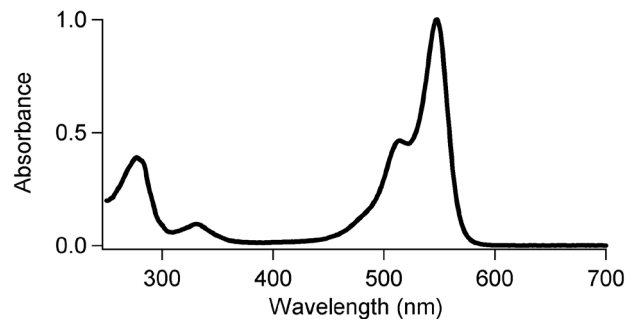
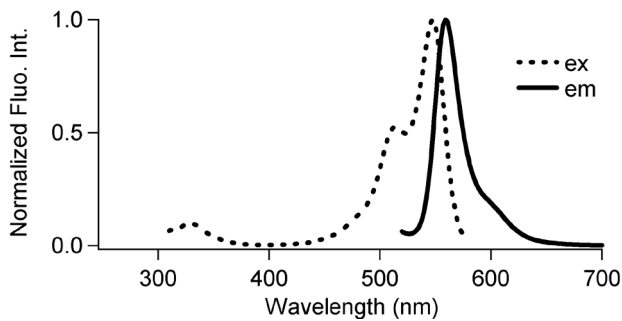


Fig 2. Schematic of the cell cycle specific fluorescence of Fucci-G₁ Orange.

CoralHue[®] mKO2: 218 amino acids

	Excit./Emiss.Maxima (nm)	Extinction Coefficient (M ⁻¹ cm ⁻¹)	Fluorescence Quantum Yield	pH sensitivity
mKO2	551/565	63,800 (551 nm)	0.62	pK _a =5.5



Fucci-G₁ Orange

1) DNA sequence

ATGGTGAGTGTGATTAACCAGAGATGAAGATGAGGTAACAT
GGACGGCTCCGTCAATGGGCATGAGTTCACAATTGAAGGTGAAG
GCACAGGCAGACCTTACGAGGGACATCAAGAGATGACACTACGC
GTCACAATGGCCGAGGGCGGCCAATGCCTTTCGGGTTTGA
AGTGTACACAGTGTCTGTTACGGCCACAGAGTATTTACTAAAT
ATCCAGAAGAGATACCAGACTATTTCAAACAAGCATTTCTGAA
GGCCTGTGATGGGAAAGGTGTTGGAGTTCGAAGATGGTGGGTC
CGCTTCACTGAGTGCATATAAGCCTTAGAGGAAACACCTTCT
ACCACAAATCCAAATTTACTGGGGTTAACTTTCCTGCCGATGGT
CCTATCATGCAAAACCAAAGTGTGATTGGGAGCCATCAACCGA
GAAAATTACTGCCAGCGACGGAGTTCTGAAGGGTATGTTACGA
TGTACCTAAAACCTGAAGGAGCGGCAATCACAATGCCAAATG
AAGACTACTTACAAGGCGGAAAAGAGATTCTTAAAATGCCAGG
AGACCATTACATCGGCCATCGCCTCGTCAGGAAAACCGAAGGCA
ACATTACTGAGCAGGTAGAAGATGCAGTAGCTCATTACTCAATG
TTGCCTTCCAGGGCGGATATCCATCACACTGGCGGCGGCTCGA
GCCAGCCCCGCCAGGCCCGCACTCCGCGCCCCGGCTCCGCTA
CCAGTGGCAGCCGCAAGCGCGCCCGCCCGCCCGCCCGCCGGA
CGCGACCAGGCCAGGCCACCGGCCCGCAGGAGACTGCGGCTGTC
GGTGGAGGAGGTTTCCAGCCCCAGTACCCCGAGGCCCGAGACA
TCCCAGCCTGCCCTTCTCCGGGCCAGAAGATAAAGAAATCCACC
CCGGCAGCAGGTGAGCCGCCACCTGACATCCGGGCAGGACCA
GGACACCATC

2) Amino acid sequence

MVSVIKPEMKMRYMDGVSNGHEFTIEGEGTGRPYEGHQEMTLR
VTMAEGGPMPFAFDLVSHVFCYGHRVFTKYPEEIPDYFKQAFPE
GLSWERSLEFEDGGSASVSAHISLRGNTFYHKSFTGVNFPADG
PIMQNGSVDWEPSTEKITASDGLKGDVTMYLKLEGGNHKQCM
KTTYKAAKEILEMPGDHYIGHRLVRKTEGNI TEQVEDAVAHYSM
LPSQGGYPShWRPLESPARPALRAPASATSGSRKRARPPAAPG
RDQARPPARRRLRLSVDEVSSPSTPEAPDIPACPSPGQKIKKST
PAAGQPPHLTSAQDQDTI

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CoralHue[®] 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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