

Fucci (Fluorescent Ubiquitination-based Cell Cycle Indicator) series
pFucci-S/G₂/M Green (Expression vector)

Code No.
AM-V9016M

Quantity
20 µg

VECTOR DESCRIPTION:

AM-V9016M pFucci-S/G₂/M Green is a mammalian expression vector encoding **CoralHue**[®] humanized monomeric Azami-Green 1 (hmAG1) fused to a part of human Geminin (hGeminin). "Fucci" stands for Fluorescent Ubiquitination-based Cell Cycle Indicator.

Geminin is an inhibitor of the DNA replication licensing factor. It accumulates during the S, G₂, and M phases, but is degraded during G₁ phase by ubiquitin-mediated proteolysis. A part of hGeminin (1-110) is also degradable in a cell cycle dependent manner.

CoralHue[®] hmAG1 sequence is codon-optimized for higher expression in mammalian cells. **CoralHue**[®] monomeric Azami-Green 1 (mAG1) has been generated from tetrameric **CoralHue**[®] Azami-Green (AG).

SOURCE: The **CoralHue**[®] AG gene was cloned from a stony coral (*Galaxea fascicularis*).

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

PURITY: A260/A280 > 1.5

STORAGE: Stored at -20°C

SEQUENCE LANDMARKS:

Fucci-S/G₂/M Green: bases 65-1102
CMV promoter: bases 4455-5027
SV40 polyA: bases 1265-1399
Kanamycin/Neomycin resistance gene: bases 2342-3133
pUC origin: bases 3724-4364
f1 origin: bases 1362-1817
SV40 origin: bases 2158-2293

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:

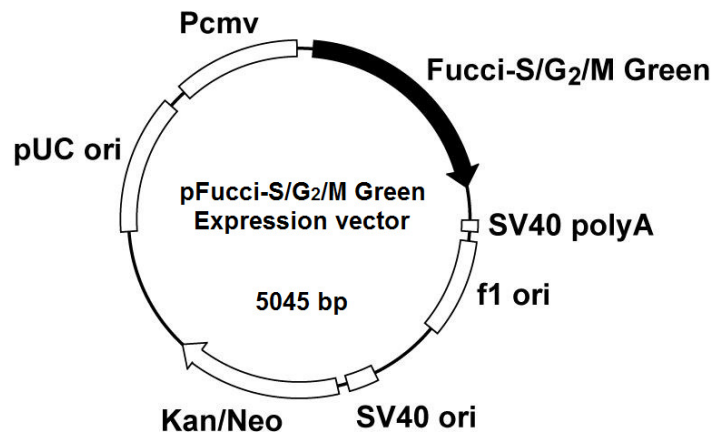
Accession Numbers: AB370333

NOTICES:

- 1) Val (encoded by GTG) is inserted as the second amino acid of **CoralHue**[®] hmAG1 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-V9014M pFucci-S/G₂/M Green (Cloning vector)
- AM-V9010M pFucci-S/G₂/M Green-Hyg (Expression vector)
- AM-V9001M pFucci-G₁ Orange (Cloning vector)
- AM-V9003M pFucci-G₁ Orange (Expression vector)
- AM-V9034M pFucci-S/G₂/M Green (N+C) (Cloning vector)
- AM-V9030M pFucci-S/G₂/M Green (N+C)-Hyg (Expression vector)



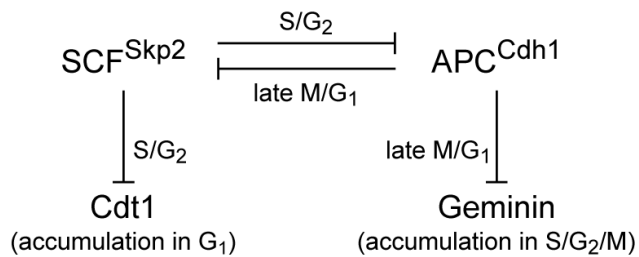


Fig 1. Cell cycle regulation by SCF^{Skp2} and APC^{Cdh1}

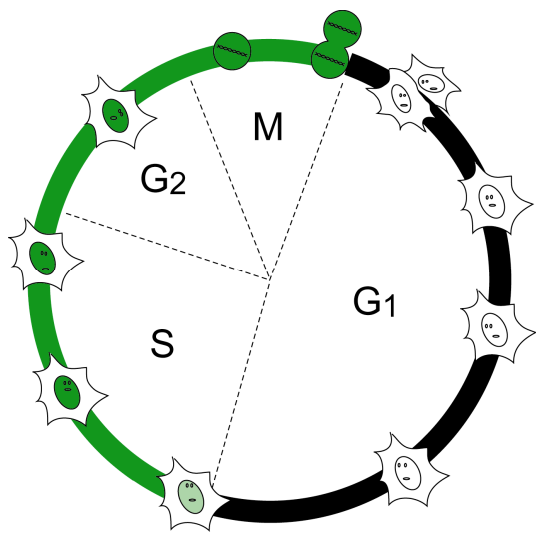


Fig 2. Schematic of the cell cycle specific fluorescence of Fucci-S/G₂/M Green.

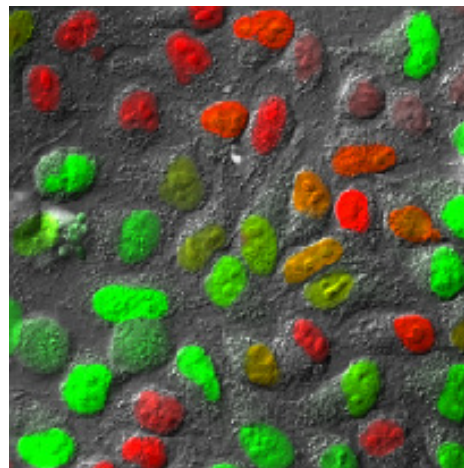
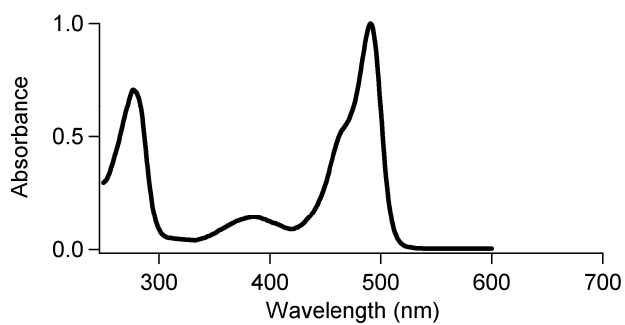
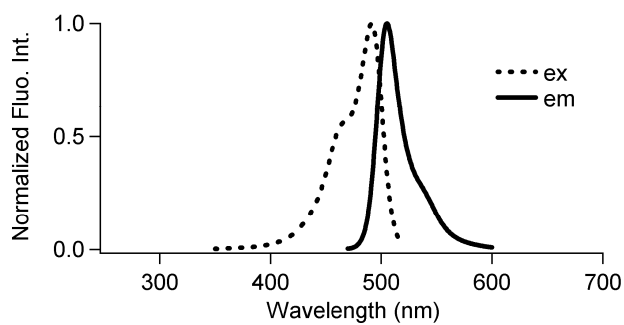


Fig 3. HeLa cells stably expressing Fucci-G₁ Orange and Fucci-S/G₂/M Green. Fucci effectively labels individual nuclei in G₁ phase orange and those in S/G₂/M phases green.

CoralHue[®] mAG1: 226 amino acids

	Excit./Emiss.Maxima (nm)	Extinction Coefficient(M ⁻¹ cm ⁻¹)	Fluorescence Quantum Yield	pH sensitivity
mAG1	492/505	55,500 (492 nm)	0.74	pK _a =5.8



Fucci-S/G2/M Green

1) DNA sequence

ATGGTGAGCGTGATCAAGCCCGAGATGAAGATCAAGCTGTGC
ATGAGGGGACCGTGAACGGCCACAACCTTCGTGATCGAGGGC
GAGGGCAAGGGCAACCCCTACGAGGGCACCCAGATCCTGGAC
CTGAACGTGACCGAGGGCGCCCCCTGCCCTTCGCCTACGAC
ATCCTGACCACCGTGTTCCAGTACGGCAACAGGGCCTTCACC
AAGTACCCCGCGACATCCAGGACTACTTCAAGCAGACCTTC
CCCGAGGGTACCCTGGGAGAGGAGCATGACCTACGAGGAC
CAGGGCATCTGCACCGCCACCAGCAACATCAGCATGAGGGGC
GACTGCTTCTTCTACGACATCAGGTTGACGGCACCAACTTC
CCCCCAACGGCCCGTGATGCAGAAGAAGACCCTGAAGTGG
GAGCCAGCACCGAGAAGATGTACGTGGAGGACGGCGTGCTG
AAGGGGACGTGAACATGAGGCTGCTGCTGGAGGGCGGGC
CACTACAGGTGCGACTTCAAGACCACCTACAAGCCAAGAAG
GAGGTGAGGCTGCCGACGCCACAAGATCGACCACAGGATC
GAGATCCTGAAGCAGACAAGGACTACAACAAGGTGAAGCTG
TACGAGAACGCCGTGGCCAGTACTCCATGCTGCCAGCCAG
GCCAAGGGATATCCATCACACTGGCGCCGCTCGAGATGAAT
CCCAGTATGAAGCAGAAACAAGAAGAAATCAAAGAGAATATA
AAGAATAGTTCTGTCCCAAGAAGAACTCTGAAGATGATTCAG
CCTTCTGCATCTGGATCTCTTGTTGGAAGAGAAAATGAGCTG
TCCGAGGCTTGTCCAAAAGGAAACATCGGAATGACCACTTA
ACATCTACAACCTCCAGCCCTGGGGTTATTGTCCCAGAATCT
AGTAAAAATAAAAATCTTGGAGGAGTCACCCAGGAGTCATTT
GATCTTATGATTAAGAAAATCCATCCTCTCAGTATTGGAAG
GAAGTGGCAGAAAAACGGAGAAAGGGCTG

2) Amino acid sequence

MVSVIKPEMKIKLCMRGTVNGHNFVIEGEGKGNPYEGTQILDNLN
VTEGAPLPFAYDILTTVFQYGNRAFTKYPADIQDYFKQTFPEGY
HWERSMTYEDQGICTATSNISMRGDCFFYDIRFDGTFPPNGPV
MQKTLKWPSTEKMYVEDGVLKGDVNMRLLEGGGHYRCDFKT
TYKAKKEVRLPDAHKIDHRIEILKHKDYNKVKLYENAVARYSM
LPSQAKGYPSHWRPLEMNPMSMKQKQEEIKENIKNSSVPRRTLKM
IQPSASGSLVGRENLSAGLSKRKHRNDHLTSTTSSPGVIVPES
SENKNLGGVTQESFDLMIKENPSSQYWKVEAEKRRKAL

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CoralHue[®] mAG1 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).

Use of **CoralHue[®] mAG1** requires a license from MBL Co., Ltd. MBL grants non-profit research organizations the right to use the product for non-commercial research purposes. For commercial entities a commercial license is required. For more information, please contact support@mbi.co.jp
Patent Nos. JP4214209, US7247449 and EP1452591.