

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

Code No.	Quantity
AM-V9010M	20 µg

VECTOR DESCRIPTION:

AM-V9010M pFucci-S/G₂/M Green-Hyg is a mammalian expression vector encoding **CoralHue™** humanized monomeric Azami-Green1 (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 AG1 (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 4659-5231
SV40 polyA: bases 1265-1399
Hygromycin resistance gene: bases 2342-3337
pUC origin: bases 3928-4568
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:

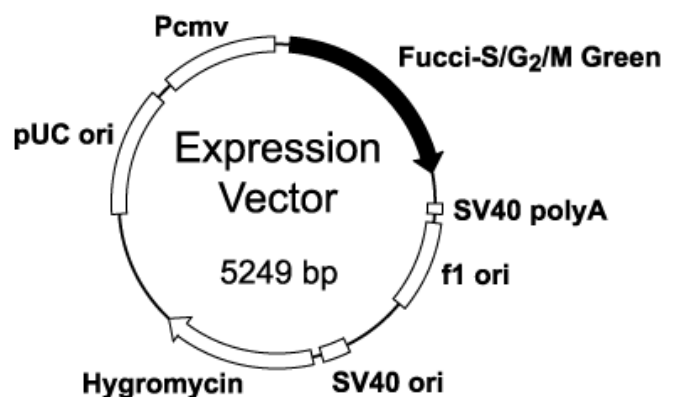
Accession Numbers: AB370333

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 hygromycin resistance gene to allow selection of stable transformants using Hygromycin B. The working concentration of Hygromycin B for mammalian cell lines varies from 50 to 1,000 µg/mL. To successfully generate a stable cell line, you need to determine the minimum concentration of Hygromycin B required to kill your untransfected host cells.
- 4) The working concentration of Hygromycin B for *E. coli*. varies from 25 to 200 µg/mL.

RELATED PRODUCTS:

AM-V9001M pFucci-G₁ Orange (Cloning vector)
AM-V9003M pFucci-G₁ Orange (Expression vector)
AM-V9014M pFucci-S/G₂/M Green (Cloning vector)
AM-V9016M pFucci-S/G₂/M Green (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)



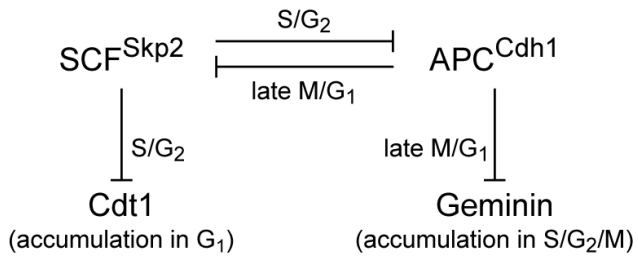


Fig 1. Cell cycle regulation by SCF^{Skp2} and APC^{Cdh1}

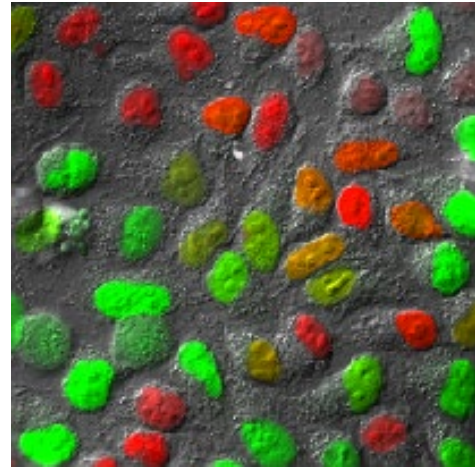


Fig 3. HeLa cells stably expressing Fucci-G₁ Orange and Fucci-S/G₂/M Green. Fucci effectively labels individual nuclei in G₁ phase orange and those in S/G₂/M phases green.

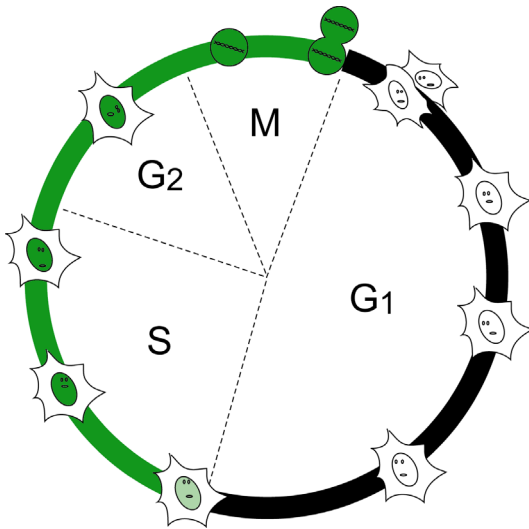
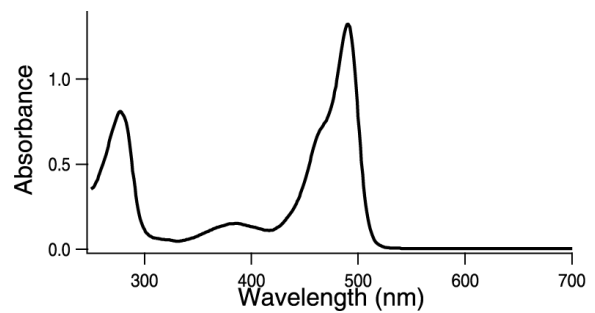
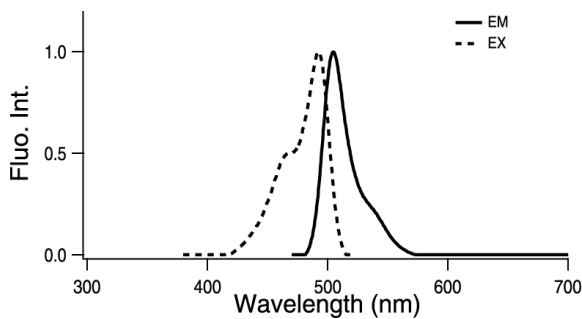


Fig 2. Schematic of the cell cycle specific fluorescence of Fucci-S/G₂/M Green.

CoralHueTM hmAG1: 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	pKa=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
CCTTCTGCATCTGGATCTCTTGTGGAAGAGAAAATGAGCTG
TCCGAGGCTTGTCCAAAAGGAAACATCGGAATGACCACTTA
ACATCTACAACCTCCAGCCCTGGGGTTATTGTCCAGAAATCT
AGTGAATAAATAATCTTGGAGGAGTCAACCAGGAGTCATTT
GATCTTATGATTAAGAAAATCCATCCTCTCAGTATTGGAAG
GAAGTGGCAGAAAACGGAGAAAGGCGCTG

2) Amino acid sequence

MVSVIKPEMKIKLCMRGTVNGHNFVIEGEGKGNPYEGTQILDNLN
VTEGAPLPFAYDILTTVFQYGNRAFTKYPADIQDYFKQTFPEGY
HWERSMTYEDQGITATSNISMRGDCFFYDIRFDGTFNPPNGPV
MQKTLKWEPESTEKMYVEDGVLKGDVNMRLLEGGGHYRCDFKT
TYKAKKEVRLPDAHKIDHRIEILKHKDYNKVKLYENAVARYSM
LPSQAKGYPSHWRPLEMNPMSMKQKQEEIKENIKNSSVPRRTLKM
IQPSASGSLVGRENELSAGLSKRKHRNDHLTSTSSPGVIVPES
SENKNLGGVTQESFDLMIKENPSSQYWKEVAEKRRKAL

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CoralHue™ mAG 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™ mAG** 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.