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

Amalgaam MBL

Fucci (*F*luorescent *U*biqutination-based *C*ell *C*ycle *I*ndicator) series

pFucci-S/G_2/M Green-Hyg (Expression vector)

Code No.	Quantity
AM-V9010M	20 µg

VECTOR DESCRIPTION:

AM-V9010M pFucci-S/G_2/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 *F*luorescent *U*biquitination-based *C*ell *C*ycle *I*ndicator.

Geminin is an inhibitor of the DNA replication licensing factor. It accumulates during the S, G_2, and M phases, but is degraded during G_1 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/G_2/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:

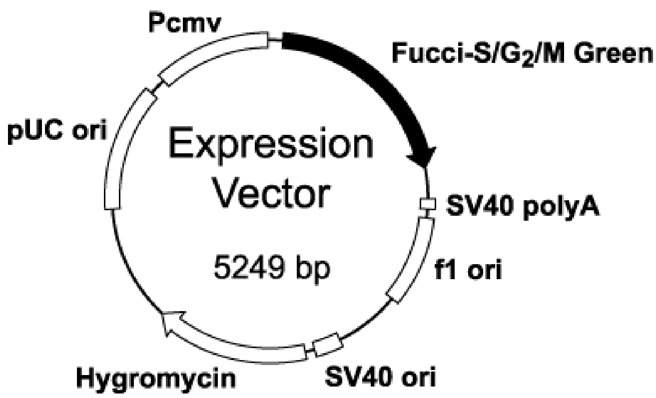
Accession Numbers: AB370333

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-G_1 Orange (Cloning vector)
AM-V9003M	pFucci-G_1 Orange (Expression vector)
AM-V9014M	pFucci-S/G_2/M Green (Cloning vector)
AM-V9016M	pFucci-S/G_2/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)



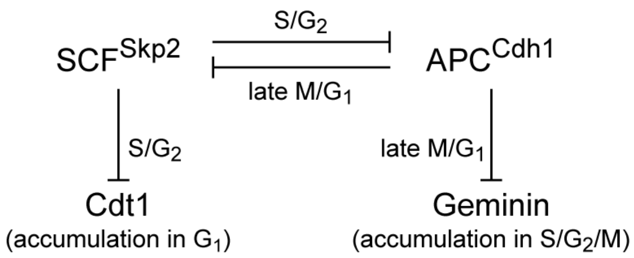


Fig 1. Cell cycle regulation by SCF^{Skp2} and APC^{Cdh1}

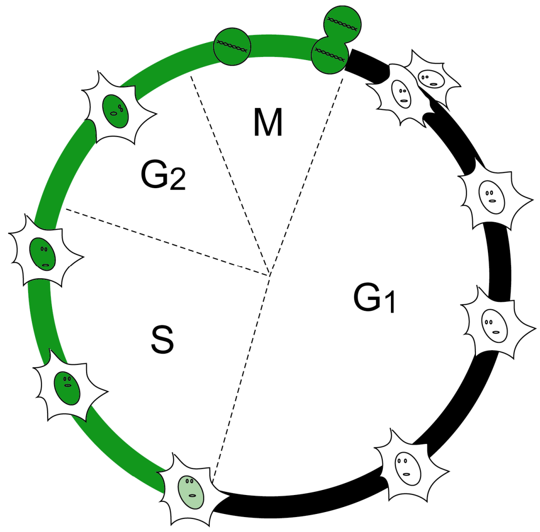


Fig 2. Schematic of the cell cycle specific fluorescence of Fucci-S/G_2/M Green.

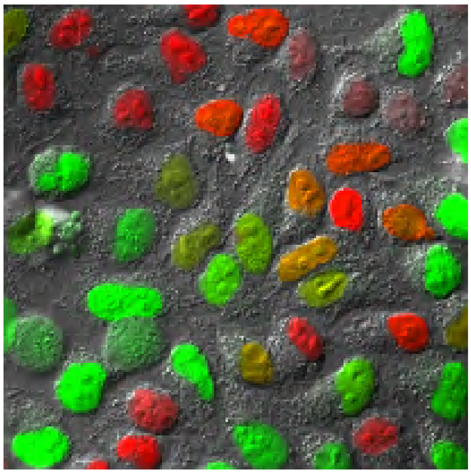
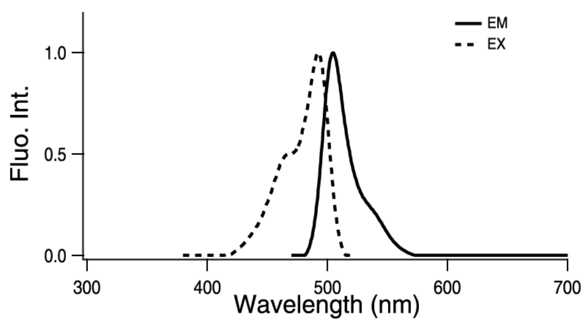
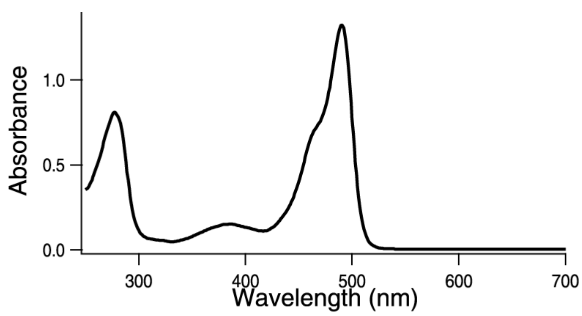


Fig 3. HeLa cells stably expressing Fucci-G_1 Orange and Fucci-S/G_2/M Green. Fucci effectively labels individual nuclei in G_1 phase orange and those in S/G_2/M phases green.

CoralHue™ **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/G_2/M Green

1) DNA sequence

ATGGTGAGCGTGATCAAGCCCGAGATGAAGATCAAGCTGTGC
ATGAGGGGCACCGTGAACGGCCACAACTTCGTGATCGAGGGC
GAGGGCAAGGGCAACCCCTACGAGGGCACCCAGATCCTGGAC
CTGAACGTGACCGAGGGCGCCCCCCTGCCCTTCGCCTACGAC
ATCCTGACCACCGTGTTCCAGTACGGCAACAGGGCCTTCACC
AAGTACCCCGCCGACATCCAGGACTACTTCAAGCAGACCTTC
CCCGAGGGCTACCACTGGGAGAGGAGCATGACCTACGAGGAC
CAGGGCATCTGCACCGCCACCAGCAACATCAGCATGAGGGGC
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CCCCCCAACGGCCCCGTGATGCAGAAGAAGACCCTGAAGTGG
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AAGGGCGACGTGAACATGAGGCTGCTGCTGGAGGGCGGCGGC
CACTACAGGTGCGACTTCAAGACCACCTACAAGGCCAAGAAG
GAGGTGAGGCTGCCCGACGCCCACAAGATCGACCACAGGATC
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TACGAGAACGCCGTGGCCAGGTACTCCATGCTGCCCAGCCAG
GCCAAGGGATATCCATCACACTGGCGGCCGCTCGAGATGAAT
CCCAGTATGAAGCAGAAACAAGAAGAAATCAAAGAGAATATA
AAGAATAGTTCTGTCCCAAGAAGAACTCTGAAGATGATTCAG
CCTTCTGCATCTGGATCTCTTGTTGGAAGAGAAAATGAGCTG
TCCGCAGGCTTGTCCAAAAGGAAACATCGGAATGACCACTTA
ACATCTACAACTTCCAGCCCTGGGGTTATTGTCCCAGAATCT
AGTGAAAATAAAAATCTTGGAGGAGTCACCCAGGAGTCATTT
GATCTTATGATTAAAGAAAATCCATCCTCTCAGTATTGGAAG
GAAGTGGCAGAAAAACGGAGAAAGGCGCTG

2) Amino acid sequence

MVSVIKPEMKIKLCMRGTVNGHNFVIEGEGKGNPYEGTQILDLN
VTEGAPLPFAYDILTTVFQYGNRAFTKYPADIQDYFKQTFPEGY
HWERSMTYEDQGICTATSNISMRGDCFFYDIRFDGTNFPPNGPV
MQKKTLKWEPSTEKMYVEDGVLKGDVNMRLLLEGGGHYRCDFKT
TYKAKKEVRLPDAHKIDHRIEILKHDKDYNKVKLYENAVARYSM
LPSQAKGYPSHWRPLEMNPSMKQKQEEIKENIKNSSVPRRTLKM
IQPSASGSLVGRENELSAGLSKRKHRNDHLTSTTSSPGVIVPES
SENKNLGGVTQESFDLMIKENPSSQYWKEVAEKRRKAL