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 (N+C)-Hyg (Expression vector)

Code No.	Quantity
AM-V9030M	20 µg

VECTOR DESCRIPTION:

AM-V9030M pFucci-S/G2/M Green (N+C)-Hyg is a mammalian expression vector encoding ***CoralHue***™ humanized monomeric Azami-Green 1 (hmAG1) fused to a part of human Geminin (hGeminin). pFucci-S/G2/M Green (N+C)-Hyg can trace the silhouette of individual cells in S/G2/M phases with fluorescence. "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, G2, and M phases, but is degraded during G1 phase by ubiquitin-mediated proteolysis. A part of hGeminin (1-60) 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/G_2/M Green(N+C): bases 65-952
CMV promoter: bases 4509-5081
SV40 polyA: bases 1115-1249
Hygromycin resistance gene: bases 2192-3187
pUC origin: bases 3778-4418
f1 origin: bases 1212-1667
SV40 origin: bases 2008-2143

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:

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GenBank:

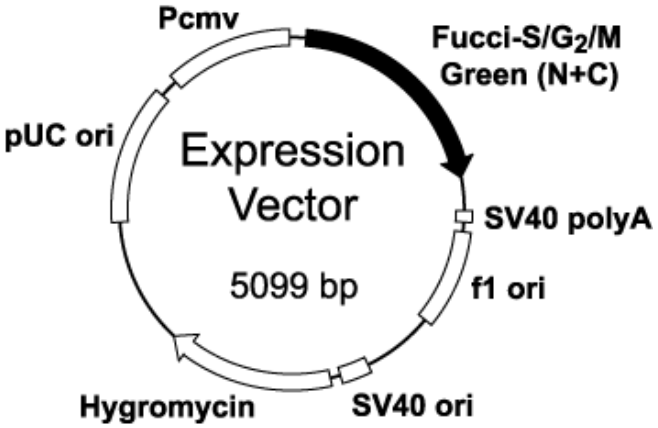
Accession Numbers: AB505861

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 1000 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-V9010M	pFucci-S/G2/M Green-Hyg (Expression vector)
AM-V9034M	pFucci-S/G2/M Green (N+C) (Cloning vector)



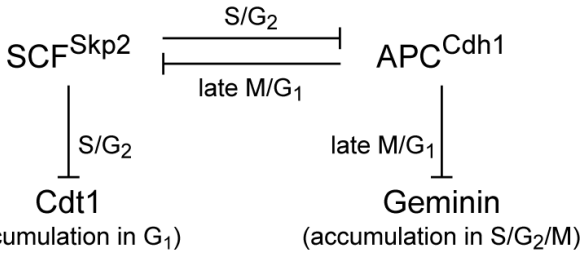


Fig 1. Cell cycle regulation by SCF^{Skp2} and APC^{Cdh1}

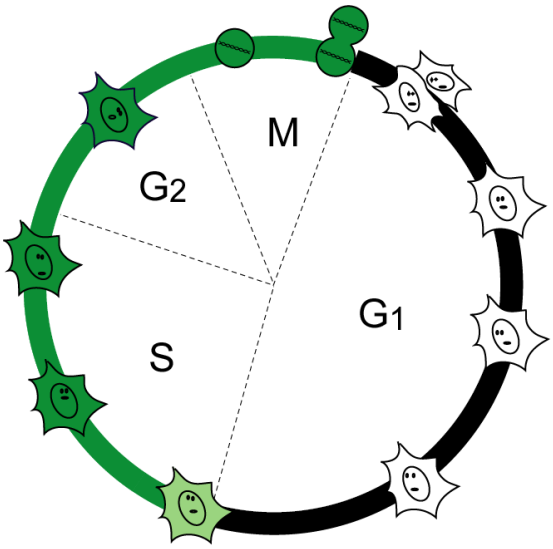


Fig 2. Schematic of the cell cycle specific fluorescence of Fucci-S/G_2/M Green (N+C).

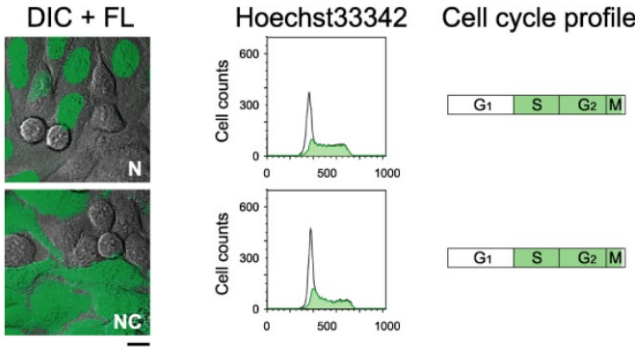
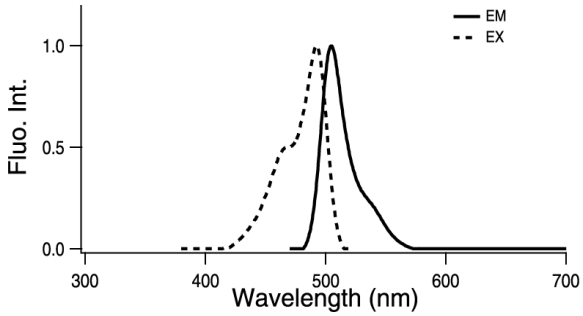
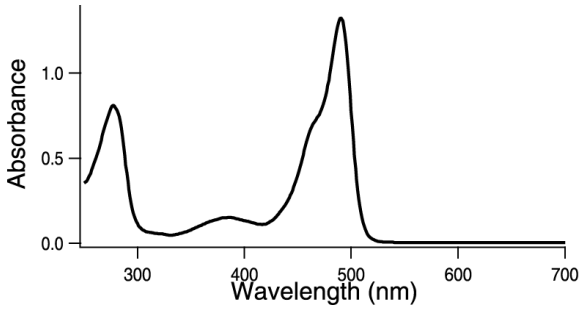


Fig 3. HeLa cells stably expressing S/G_2/M Markers.
(DIC + FL) Typical differential interference contrast (DIC) and fluorescence (FL) images of HeLa cells stably expressing constructs. Distribution patterns are indicated as follows: N, nucleus; C, cytosol; NC, nucleus and cytosol. Scale bar, 10 μm.
(Hoechst33342) HeLa cells stably expressing S/G2/M markers were stained with Hoechst33342 and analyzed using fluorescence-activated cell sorting.
(Cell cycle profile) Cell cycle phases highlighted by fluorescence are colored.

***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/G2/M Green (N+C) DNA sequence

ATGGTGAGCGTGATCAAGCCCGAGATGAAGATCAAGCTGTGC
ATGAGGGGCACCGTGAACGGCCACAACTTCGTGATCGAGGGC
GAGGGCAAGGGCAACCCCTACGAGGGCACCCAGATCCTGGAC
CTGAACGTGACCGAGGGCGCCCCCCTGCCCTTCGCCTACGAC
ATCCTGACCACCGTGTTCCAGTACGGCAACAGGGCCTTCACC
AAGTACCCCGCCGACATCCAGGACTACTTCAAGCAGACCTTC
CCCGAGGGCTACCACTGGGAGAGGAGCATGACCTACGAGGAC
CAGGGCATCTGCACCGCCACCAGCAACATCAGCATGAGGGGC
GACTGCTTCTTCTACGACATCAGGTTCGACGGCACCAACTTC
CCCCCCAACGGCCCCGTGATGCAGAAGAAGACCCTGAAGTGG
GAGCCCAGCACCGAGAAGATGTACGTGGAGGACGGCGTGCTG
AAGGGCGACGTGAACATGAGGCTGCTGCTGGAGGGCGGCGGC
CACTACAGGTGCGACTTCAAGACCACCTACAAGGCCAAGAAG
GAGGTGAGGCTGCCCGACGCCCACAAGATCGACCACAGGATC
GAGATCCTGAAGCACGACAAGGACTACAACAAGGTGAAGCTG
TACGAGAACGCCGTGGCCAGGTACTCCATGCTGCCCAGCCAG
GCCAAGGGATATCCATCACACTGGCGGCCGCTCGAGATGAAT
CCCAGTATGAAGCAGAAACAAGAAGAAATCAAAGAGAATATA
AAGAATAGTTCTGTCCCAAGAAGAACTCTGAAGATGATTCAG
CCTTCTGCATCTGGATCTCTTGTTGGAAGAGAAAATGAGCTG
TCCGCAGGCTTGTCCAAAAGGAAACATCGGAATGACCACTTA
ACATCT

Fucci-S/G2/M Green (N+C) amino acid sequence

MVSVIKPEMKIKLCMRGTVNGHNFVIEGEGKGNPYEGTQILDLN
VTEGAPLPFAYDILTTVFQYGNRAFTKYPADIQDYFKQTFPEGY
HWERSMTYEDQGICTATSNISMRGDCFFYDIRFDGTNFPPNGPV
MQKKTLKWEPSTEKMYVEDGVLKGDVNMRLLLEGGGHYRCDFKT
TYKAKKEVRLPDAHKIDHRIEILKHDKDYNKVKLYENAVARYSM
LPSQAKGYPSHWRPLEMNPSMKQKQEEIKENIKNSSVPRRTLKM
IQPSASGSLVGRENELSAGLSKRKHRNDHLTS