Fluorescent Proteins

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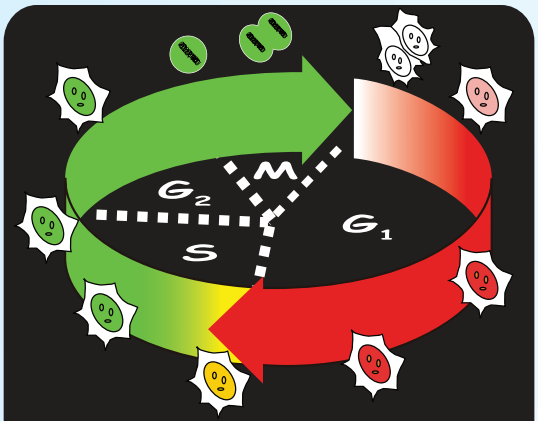
Real time visualization of the cell cycle

FUCCI

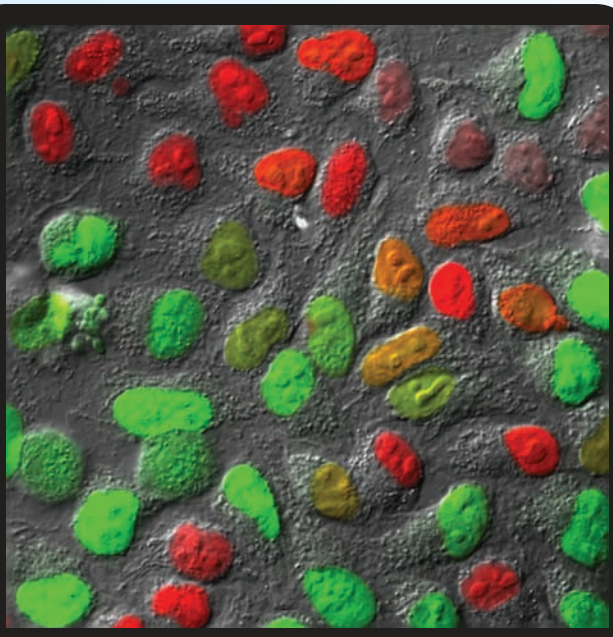
(Fluorescent Ubiquitination-based Cell Cycle Indicator)



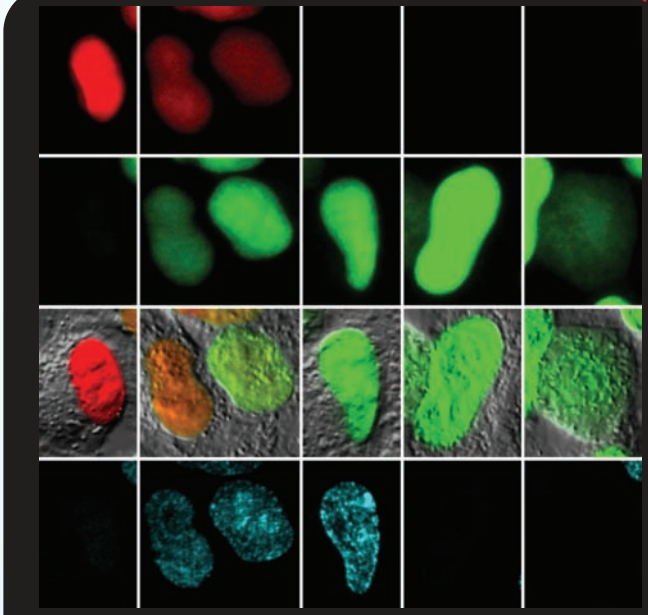
Visualizing the cell cycle: Schematic representation of the fluorescence observed in Fucci-transfected cells over the cell cycle phases. An orange fluorecent protein marks nuclei in G_1 (shown in red), while a green fluorescent protein marks nuclei in S, G_2 and M in green.

- **Used for imaging the spatio-temporal patterns of cell cycle dynamics**
- **FACS - Sort your cells by cell cycle phase**
- **In Vivo analysis**
- **Areas of Research:**
  Cell Growth, Differentiation, Development, Regeneration and Carcinogenisis

NEW



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 phase green.



Typical fluorescence images in HeLa cells expressing Fucci-G_1 Orange and Fucci-S/G_2/M Green and immunofluorescence for incorporated BrdU at G_1, G_1/S, S, G_2, and M phases.

Images courtesy of:
Dr.Asako Sakaue-Sawano and Dr.Atsushi Miyawaki
Laboratory for Cell Function and Dynamics, Advanced Technology Development Group, Brain Science Institute, RIKEN; Life Function Dynamics, ERATO, JST
These images were obtained using the stable cell line is Reference (Sakaue-Sawano, A., et al., Cell 132, 487-498 (2008)), obtained with modification of Amalgaam products. For details please refer to the Reference given.

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Fucci

Fucci (Fluorescent Ubiqutination-based Cell Cycle Indicator)is a set of fluorescent probes which enable the visualization of cell cycle progression in living cells. Fucci takes advantage of the fact that the replication licensing factors Cdt1 and Geminin are only present during specific phases of the cell cycle. A fusion protein of a fragment of Cdt1 (amino acids 30-120) with the fluorescent protein monomeric Kusabira-Orange 2 (mKO2) serves as an indicator of G_1 phase, while a fusion protein of a fragment of Geminin (amino acids 1-120) with the fluorescent protein monomeric Azami-Green 1 (mAG1) visualizes S, G_2 and M phase. The cell cycle indicator takes advantage of the highly selective, rapid degradation of the replication licensing factors, mediated by the ubiquitin-proteasome system.

By visualizing the cell cycle, Fucci is a powerful tool to investigate any process that has to do with cell growth and differentiation, such as the development and regeneration of organs as well as carcinogenesis.

Terms:

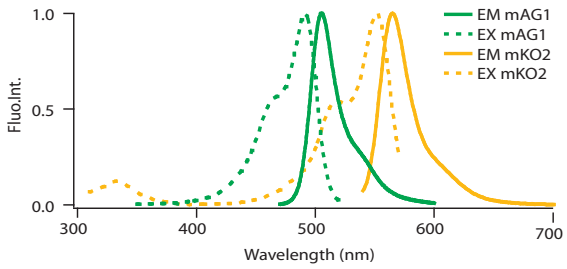
Cdt1: Cdc 10 dependent transcript 1 is a conserved replication factor required in licensing the chromosome for a single round of DNA synthesis. Abundantly expressed throughout the cell cycle, Cdt1 is ubiquinated by the ubiquitin ligase complex SCF^{Skp2} during S and G_2 phase and degraed by the proteasome.

Geminin: Geminin inhibits the licensing activity of Cdt1. Geminin interferes with the binding of licensing factors to the origin of replication once a chromosome has started to replicate during S phase. During M and G_1 phase, geminin is ubiquitinated by the ubiquitin ligase complex APC^{Cdh1} and degraded by the proteasome.

Fluorescence characteristics

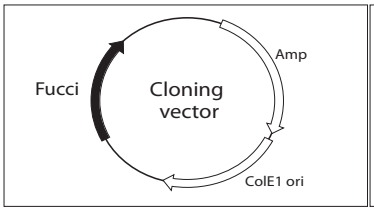
	Excitation/ Emission Maxima	Excitation Coefficient ($M^{-1}cm^{-1}$)	Fluorescence Quantum Yield	pH sensitivity
mAG1	492/505	55,500 (492 nm)	0.74	pKa=5.8
mKO2	551/565	63,800 (551 nm)	0.62	pKa=5.5

Spectra of Fucci probes for Cell Cycle Analysis

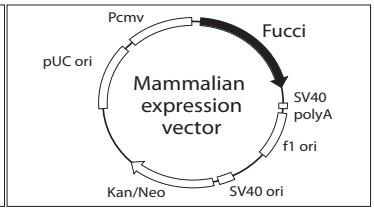


Excitation (dotted line) and emission (solid line) spectra pf mAG, mKO2

Vector



pFucci-G_1 Orange (AM-V9001)
pFucci-S/G_2/M Green (AM-V9014)



pFucci-G_1 Orange (AM-V9003)
pFucci-S/G_2/M Green (AM-V9016)

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)

Code No.	Description	Size
AM-V9001	pFucci-G_1 Orange (cloning vector)	20 μg
AM-V9003	pFucci-G_1 Orange (expression vector)	20 μg
AM-V9014	pFucci-S/G_2/M Green (cloning vector)	20 μg
AM-V9016	pFucci-S/G_2/M Green (expression vector)	20 μg
AM-VS0601	Fucci Set (AM-V9001 + AM-V9014)	20 μg + 20 μg
AM-VS0602	Fucci Set (AM-V9003 + AM-V9016)	20 μg + 20 μg
AM-VS0603	Fucci Set (AM-V9001 + AM-V9016)	20 μg + 20 μg
AM-VS0604	Fucci Set (AM-V9003 + AM-V9014)	20 μg + 20 μg

This product is licensed from RIKEN and Tokyo Metropolitan Institute of Medical Science.
CoralHue proteins were co-developed with the Laboratory for Cell Function and Dynamics, the Advanced Technology Development Center, the Brain Science Institute fo Physical and Chmical Research (RIKEN) (lab head Dr. Atsushi Miyawaki).