



FUCCI

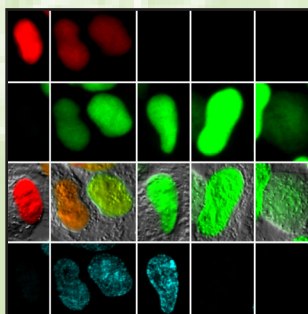
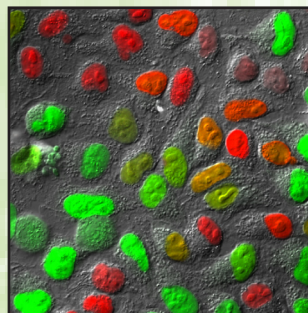
Fluorescence Ubiquitination-
based Cell Cycle Indicator

- Used for imaging the spatiotemporal 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 Carcinogenesis

Real Time Visualization of the Cell

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 phase green.

Typical fluorescence images in HeLa cells expressing Fucci-G₁ Orange and Fucci-S/G₂/M Green and immunofluorescence for incorporated BrdU at G₁, G₂/S, S, G₂, and M phases.



Images courtesy of:

Dr. Asako Sakaue-Sawano and Dr. Atushi 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 in 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 Ubiquitination-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 G₁ 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, G₂ 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:

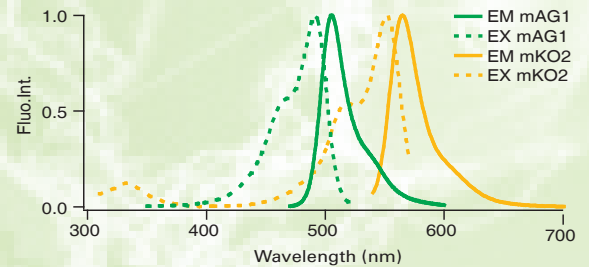
Cdt1: Cdc 10 dependent transcript 1 is a conserved replication factor required in licensing the chromosome for a single round of DNA synthesis. Abundantly expressed throughout the cell cycle, Cdt1 is ubiquitinated by the ubiquitin ligase complex SCF^{Skp2} during S and G₂ phase and degraded by the proteasome.

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 G₁ phase, geminin is ubiquitinated by the ubiquitin ligase complex APC^{Cdh1} and degraded by the proteasome.

Fluorescence characteristics

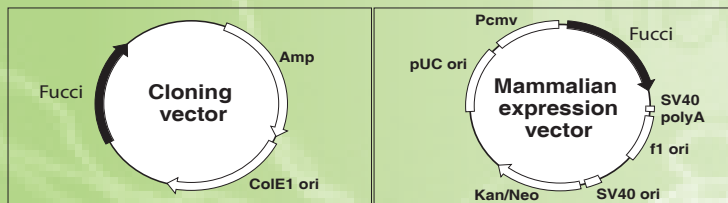
	Excitation/ Emission Maxima	Excitation Coefficient (M ⁻¹ cm ⁻¹)	Fluorescence Quantum Yield	pH sensitivity
mAG1	492/505	55,500 (492 nm)	0.74	pKa=5.8
mKO2	551/565	63,800 (551 nm)	0.62	pKa=5.5

Spectra of Fucci probes for Cell Cycle Analysis



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

Vector



pFucci-G₁ Orange (AM-V9001)
pFucci-S/G₂/M Green (AM-V9014)

pFucci-G₁ Orange (AM-V9003)
pFucci-S/G₂/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 μ
AM-V9003	pFucci-G ₁ Orange (expression vector)	20 μ
AM-V9014	pFucci-S/G ₂ /M Green (cloning vector)	20 μ
AM-V9016	pFucci-S/G ₂ /M Green (expression vector)	20 μ
AM-V9030	pFucci-S/G ₂ /M Green(N+C)-Hyg(expression vector)	20 μ
AM-V9034	pFucci-S/G ₂ /M Green (N+C) (Cloning vector)	20 μ
AM-VS0601	CoralHue® Fucci Cloning vector set (G1 Orange + S/G2/M Green)	20 μ + 20 μ
AM-VS0605	CoralHue® Fucci Cloning vector set (G1 Orange + S/G2/M Green(N+C))	20 μ + 20 μ
AM-VS0607	CoralHue® Fucci Expression vector set (G1 Orange + S/G2/M Green-Hyg)	20 μ + 20 μ
AM-VS0608	CoralHue® Fucci Expression vector set (G1 Orange + S/G2/M Green(N+C)-Hyg)	20 μ + 20 μ

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 for Physical and Chemical Research (RIKEN) (lab head Dr. Atsushi Miyawaki).