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Image based Protein-Protein interaction analysis

Fluoppi

Fluoppi：Ash-hAG

[mTOR-FKBP12]

Code: AM-8202M

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1. Introduction

Fluoppi is a technology providing an easy way to visualize protein-protein interactions (PPIs) with a high signal to noise ratio. It employs an oligomeric assembly helper tag (Ash-tag) and a tetrameric fluorescent protein tag (FP-tag) to create detectable fluorescent foci when there are interactions between two proteins fused to the tags. By way of example, genetic fusion of protein X with FP-tag, and Y with Ash-tag creates a tetrameric fluorescent fusion protein X-FP and an oligomeric fusion protein Y-Ash respectively (Fig. 1). Because each fusion protein has multiple Xs or Ys, interaction between X and Y causes large lattice like complexes where the fluorescence by X-FP is concentrated and detectable as fluorescent foci (Fig. 2).

Interaction between FRB domain of mTOR and FKBP12 is well studied as an experimental model of PPI, because it can be induced simply by adding a cell permeable small molecule, Rapamycin (Choi J, *et al.* 1996).

Fluoppi : Ash-hAG [mTOR-FKBP12] contains two plasmids for detecting mTOR(FRB)-Rapamycin-FKBP12 ternary complex in living cells. One encodes a fusion protein of Ash/FKBP12, and the other encodes hAG/mTOR(2025-2114) . Co-transfection of pAsh/FKBP12 and phAG/mTOR results in an evenly disseminated fluorescence in scales throughout the transfected cell. After addition of rapamycin, fluorescent small foci appear gradually in several minutes, indicating the mTOR(FRB)-Rapamycin-FKBP12 complex induced by Rapamycin.

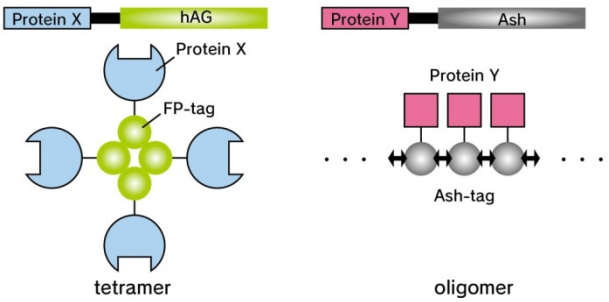


Figure 1 | Key components of Fluoppi technology

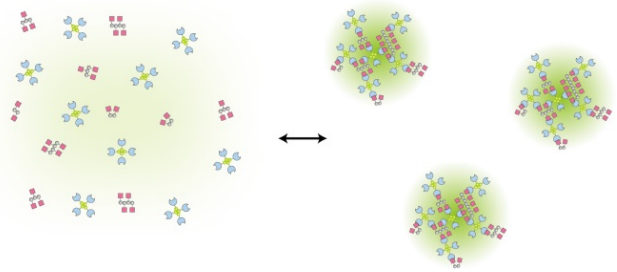


Figure 2 | Mechanism of action

2. Product components and Storage Condition

Plasmids	Vial color	Form
pAsh/FKBP12	White	10 μg: Dry form
phAG/mTOR	White	10 μg: Dry form

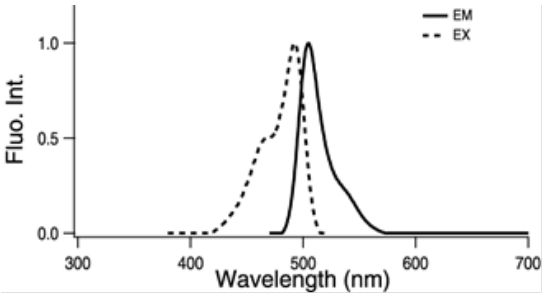
Reconstitution in 10-50 μL of sterilized distilled water.
Storage condition: Store at -20°C. Reconstituted solution should be kept at -20°C.

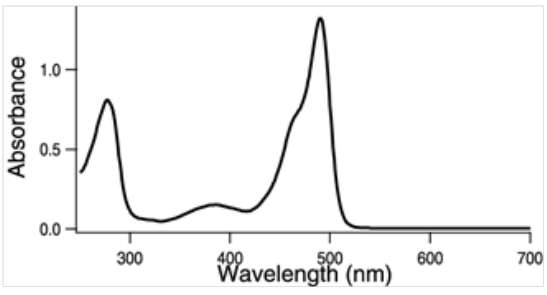
3. Additional Materials Required

- Cell culture related materials (Mammalian cells, Cell culture medium, Cell culture dish, Plate)
- Transfection reagent
- Buffer for imaging (HBSS, PBS, Good's Buffer)
- Fluorometric detector (Fluorescence microscopy, Plate imager)

4. Properties of Fluorescent protein "hAG"

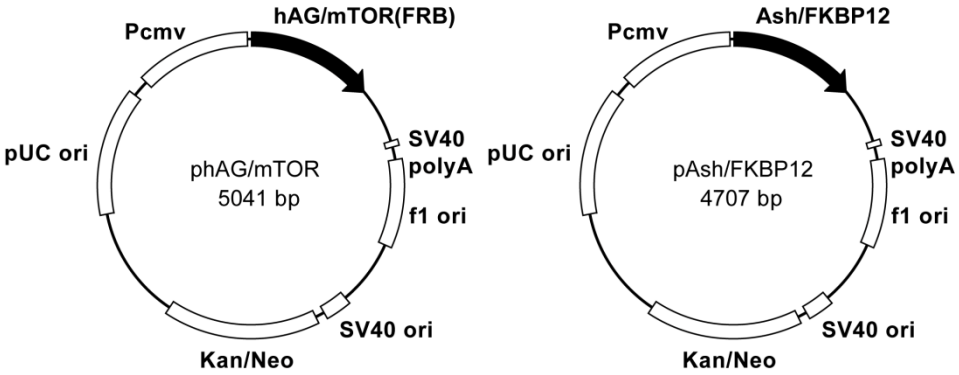
CoralHue® humanized Azami-Green (hAG), cloned from the stony coral (azami-sango in Japan), absorbs light maximally at 492 nm and emits green light at 505 nm. hAG forms tetramer and is featured by its fast maturation and highly photo and pH stable nature. The gene codon is optimized for mammalian cells.





Fluorescent protein	Excitation/Emission maximum (nm)	Extinction coefficient ($M^{-1}cm^{-1}$)	Fluorescence quantum yield	pKa
hAG	492/505	72,300 (492 nm)	0.67	<5.0

5. Plasmid Maps



6. Example of Procedure

[Transfection]

HeLa-S3 Cells were grown in DMEM (Sigma No. D6046) supplemented with 10% fetal bovine serum (FBS) and 1% Pen Strep (Sigma No. P4333) at 37℃ in 5% CO_2 atmosphere. Cells were plated in 8 well Lab-Tek Chambered Coverglass (Thermo Scientific No. 155411) at 2×10^4 cells per well with 0.3 ml medium. After incubation for 16 hours, cells were transiently transfected with a pair of plasmid DNAs (0.1 µg each) using TurboFect (Thermo Scientific, 0.3 µl) and incubated for another 20 to 24 hours. Then cells were subjected to analysis.

[Imaging]

A wide field fluorescence microscopy was used to observe PPI. Excitation of hAG fluorescence was performed by using a 75-W Xenon lamp with a BP460-480HQ filter (Olympus). Emitted light was detected by an ORCA-ER CCD camera (Hamamatsu Photonics) with a BA495-540HQ band pass filter (Olympus) and a 485 nm dichroic mirror (Olympus). MetaMorph software (Molecular Devices) was used for data collections and analysis.

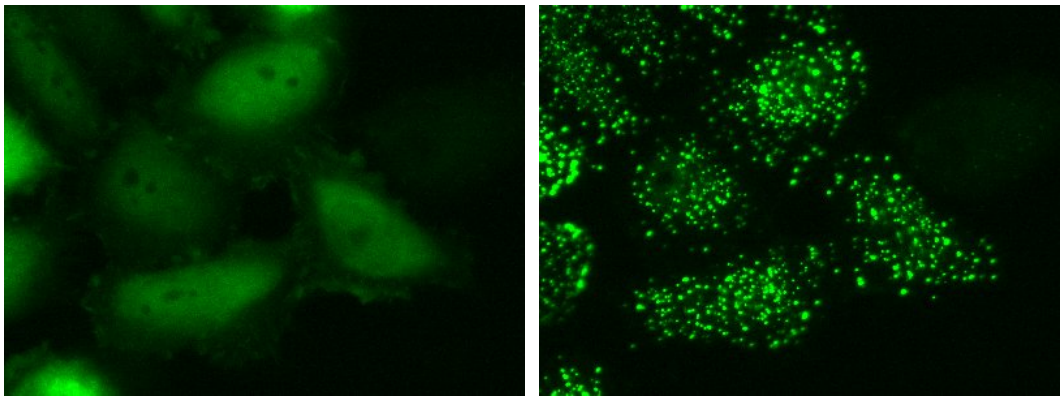


Figure 3 | HeLa-S3 cells transiently expressing both Ash/FKBP12 and hAG/mTOR(FRB) were observed at 0 minute (left) and 12 minutes (right) after addition of 500 nM Rapamycin.

7. Reference

Choi J, et al., Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. Science 273, 239-242 (1996) [PMID: 8662507]

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9. Related products

AM-8011M	Fluoppi Ver.2 : Ash-hAG (Ash-MNL/MCL + hAG-MNL/MCL)
AM-8012M	Fluoppi Ver.2 : Ash-Red (Ash-MNL/MCL + Monti-Red-MNL/MCL)
AM-8201M	Fluoppi : Ash-hAG [p53-MDM2]
AM-8202M	Fluoppi : Ash-hAG [mTOR-FKBP12]
AM-VS0801M	humanized Azami-Green for Fluoppi (phAG-MNL/MCL)
AM-VS0802M	Monti-Red for Fluoppi (pMonti-Red-MNL/MCL)