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Image based Protein-Protein interaction analysis

Fluoppi

Fluoppi : Ash-hAG

[p53-MDM2]

Code: AM-8201M

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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).

p53 is well known as a tumor suppressor protein inducing cell cycle arrest or apoptosis when cells are exposed to a variety of cellular stresses. An E3 ubiquitin ligase, MDM2, directly interacts with and ubiquitinates p53, and the subsequent product, ubiquitinated p53, is targeted for degradation by proteasome. Over-expression of MDM2 as well as its gene amplification are observed in a variety of cancers. Therefore, small molecule inhibitors of p53-MDM2 interaction have been developed in several clinical trials for cancer therapy.

Fluoppi : Ash-hAG [p53-MDM2] contains two plasmids for detecting p53-MDM2 interaction in living cells. One encodes a fusion protein Ash/p53(1-70), and the other encodes hAG/MDM2(1-119) . Co-transfection of pAsh/p53 and phAG/MDM2 results in formation of cytoplasmic fluorescent foci in scales throughout the transfected cell. After addition of p53-MDM2 PPI inhibitors, the foci disappear gradually in several minutes, indicating the p53-MDM2 complex is disrupted.

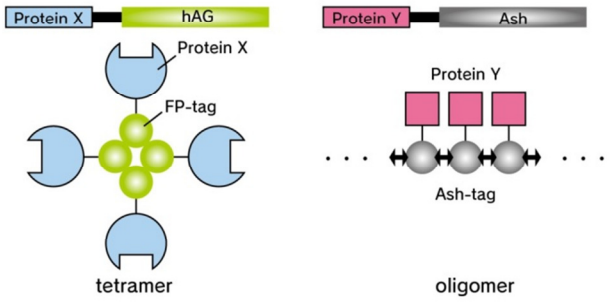


Figure 1 |Key components of Fluoppi technology

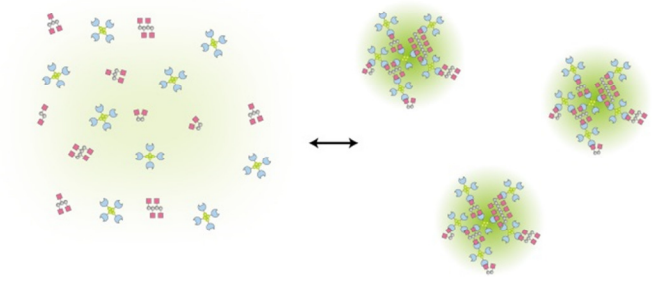


Figure 2 | Mechanism of action

2. Product Components and Storage Condition

Plasmids	Vial color	Form
pAsh/p53	White	10 μg: Dry form
phAG/MDM2	White	10 μg: Dry form

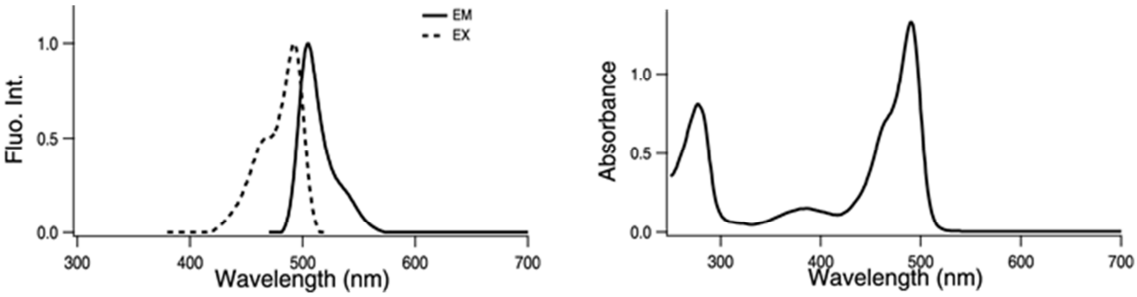
Reconstitution in 10-50 μL of sterilized distilled water.
Storage condition: Store at -20°C. Reconstituted solution should be kept at -20°C.

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)

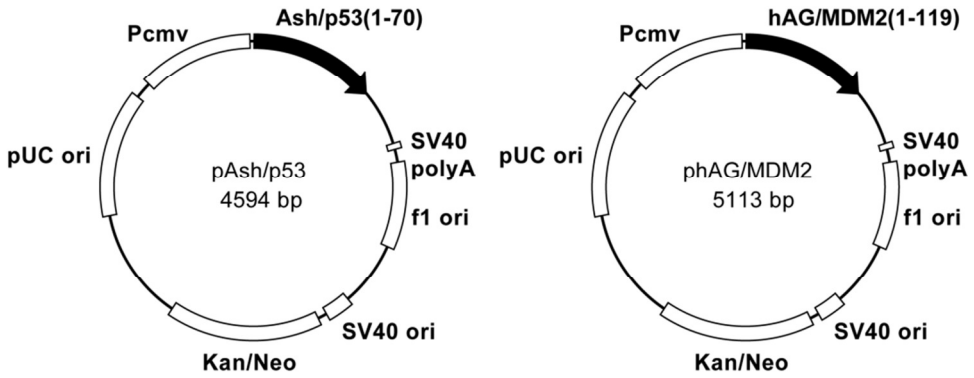
4. Properties of Fluorescent protein "hAG"

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 collagen (KOKEN No. IAC-30) coated 35 mm glass bottom dishes (IWAKI No. 3911-035) at $1x10^5$ cells per dish with 2 ml medium. After incubation for 16 hours, cells were transiently transfected with a pair of plasmid DNAs (both 1 μg) using Polyfect (QIAGEN, 10 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.

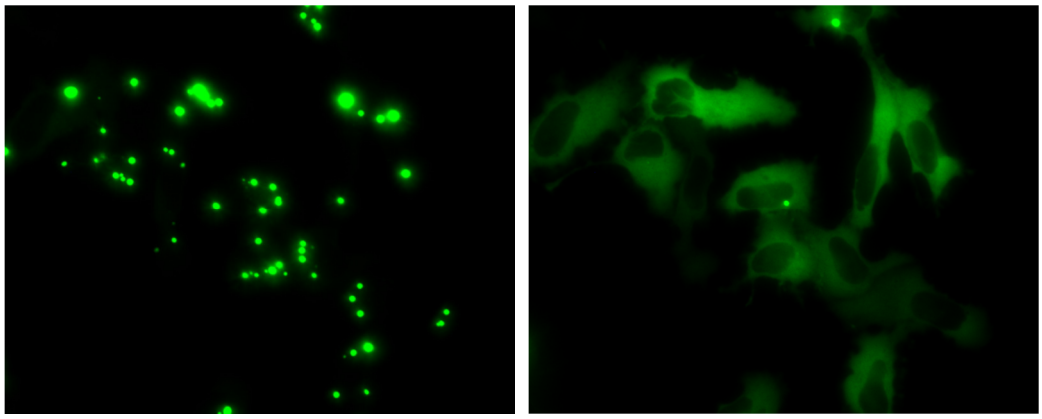


Figure 3 | HeLa-S3 cells transiently expressing both Ash/p53 and hAG/MDM2 were observed at 0 minute (left) and 15 minutes (right) after addition of 10 μM Nutlin-3*. The interactions were observed as fluorescent foci (left), and disruptions of the PPI by Nutlin-3 resulted in a cytoplasmic evenly distribution of fluorescence (right).

*Nutlin-3 is a cell permeable small molecule inhibitor of p53-MDM2 interaction (Vassilev LT, et al. 2004).

7. Reference

Vassilev LT, et al., In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science. 303, 844-848. (2004) [PMID: 14704432]

8. License

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9. Related products

AM-8001M	Fluoppi : Ash-hAG (Ash-MNL/MCL + hAG-MNL/MCL)
AM-8002M	Fluoppi : 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)