Amalgaam

MBL

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Fluorescent protein-protein interaction visualization

Fluoppi

PPIs Detection Reagent : Fluoppi [Mcl1-BAX]

Code: AM-P0007

Table of contents

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 puncta when there are interactions between two proteins fused to the tags. Schematic images are illustrated in Figure 1, where 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. Because each fusion protein has multiple Xs or Ys, the interaction between X and Y causes phase-separated droplets where the fluorescence by X-FP is concentrated and detectable as fluorescent puncta (Fig. 2).

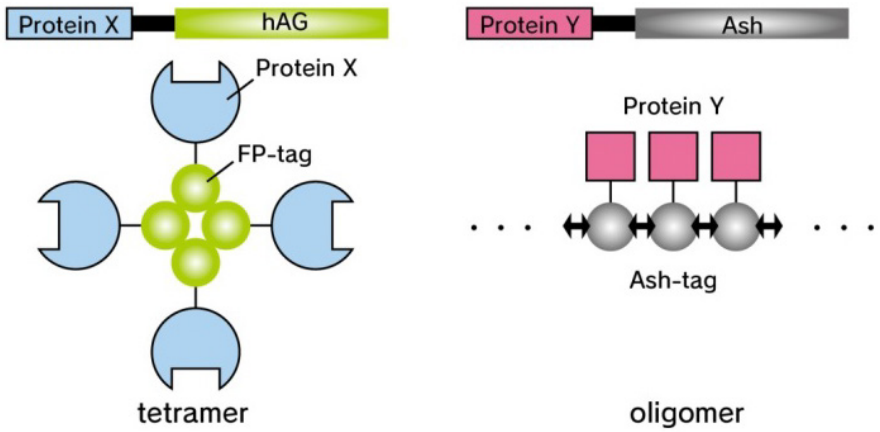


Figure 1 |Key components of Fluoppi technology

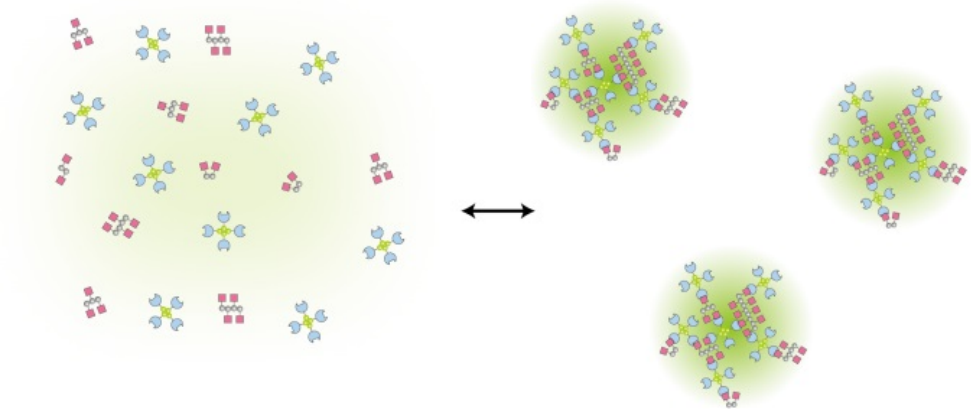


Figure 2 | Mechanism of action

2. Fluoppi : Ash-hAG [Mcl1-BAX]

This product contains two expression cassettes for detecting Mcl1-BAX interaction in living cells. One encodes a fusion protein Ash/Mcl1, and the other encodes humanized Azami-Green (hAG)/BAX. Partial sequences responsible for this interaction are used for this product. Co-transfection of DNA cassettes, Ash/Mcl1 and hAG/BAX, results in formation of cytoplasmic fluorescent puncta. After addition of Mcl1-BAX PPI inhibitors, the puncta disappeared within 30 minutes, indicating the Mcl1-BAX complex was disrupted.

3. Product Components and Storage Condition

DNA cassettes	Amount:	Form
Ash/Mcl1	10 μg	Dry form
hAG/BAX	10 μg	Dry form

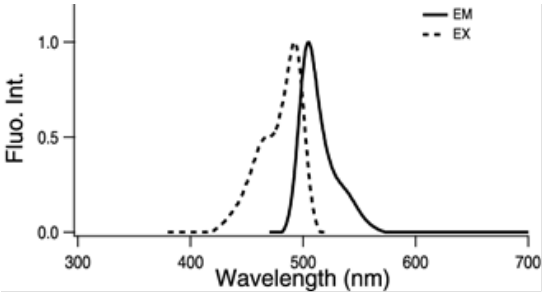
Reconstitute in 10-50 μL of sterilized distilled water before use.
Storage condition: Store at -20°C. Reconstituted solution should be kept at -20°C.

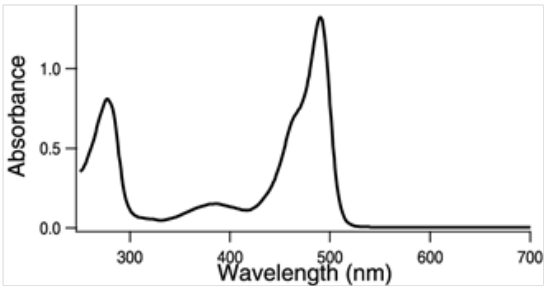
4. Additional Materials Required

- Cell culture related materials (Mammalian cells, Cell culture medium, Cell culture dish, etc.)
- Transfection reagents or equipment.
- Buffer for imaging (HBSS, PBS, Good's Buffers, etc.)
- Fluorometric detector (Fluorescence microscopy or Plate imager)

5. Properties of Fluorescent protein “Azami-Green”

humanized Azami-Green (hAG), cloned from the stony coral (Azami-sango in Japanese), 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.

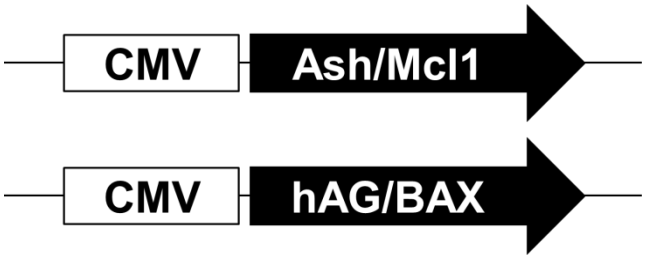




Fluorescent protein	Excitation/Emission maximum (nm)	Extinction coefficient ($M^{-1}cm^{-1}$)	Fluorescence quantum yield	pKa
hAG	492/505	72,300 (492 nm)	0.67	<5.0

6. Expression Cassettes

Both open reading frames are driven by the CMV promoter in mammalian cells.



7. Example of Procedure

[Transfection]

HEK293 cells were grown in DMEM (Sigma; code No. D5796) supplemented with 10% fetal bovine serum (FBS) and 1% Pen Strep (Gibco; code No. 15140-122) at 37°C in 5% CO_2 atmosphere. Cells were plated in collagen (KOKEN; code No. IAC-30) coated Lab-Tek Chambered Coverglass (Nunc; code No.155411) at $2x10^4$ cells per well with 200 µL medium. After incubation for 16 hours, cells were transiently transfected with a pair of plasmid DNAs (both 200 ng) diluted in 10 µL of Opti-MEM® (Gibco; code No. 31985-070) using FuGENE® HD Transfection Reagent (Promega, 0.8 µL). After incubation for another 20 to 24 hours, cells were subjected to analysis.

[Imaging]

A wide field fluorescence microscopy was used to observe PPI. Excitation of hAG fluorescence was performed by a 75-W Xenon lamp with a BP460-480HQ filter (Olympus). Emitted light was detected by an ORCA-Flash4.0 sCMOS 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.

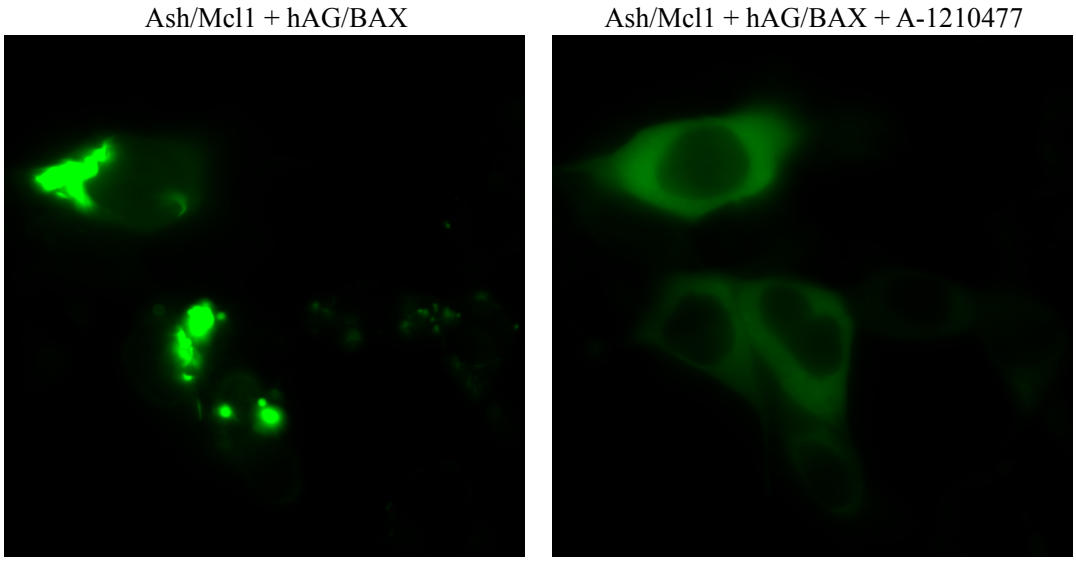


Figure 3 | HEK293 cells transiently expressing both Ash/Mcl1 and hAG/BAX were observed before (left) and 30 minutes after addition of 25 µM A-121047* (right). The interactions were observed as fluorescent puncta (left), and disruptions of the PPI by A-121047 resulted in cytoplasmic diffused distribution of fluorescence (right).

* A Mcl1 inhibitor. (Leverson, JD., *et al*. 2015).

8. References

Watanabe T, *et al*., Genetic visualization of protein interactions harnessing liquid phase transitions. Sci Rep. 7, Article number: 46380 (2017) [PMID: 28406179]

Leverson JD, *et al*., Potent and selective small-molecule MCL-1 inhibitors demonstrate on-target cancer cell killing activity as single agents and in combination with ABT-263. Cell Death Dis. 6:e1590. (2015) [PMID: 25590800]

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)

10. Notice to Purchaser

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