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Fluorescent protein-protein interaction visualization

Fluoppi[†]

PPIs Detection Reagent : Fluoppi [Bcl2-BAK]

Code: AM-P0004

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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 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).

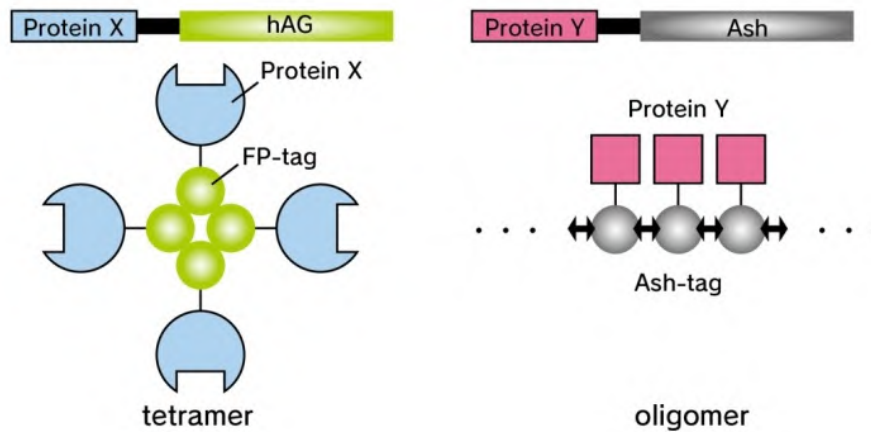


Figure 1 | Key components of Fluoppi technology

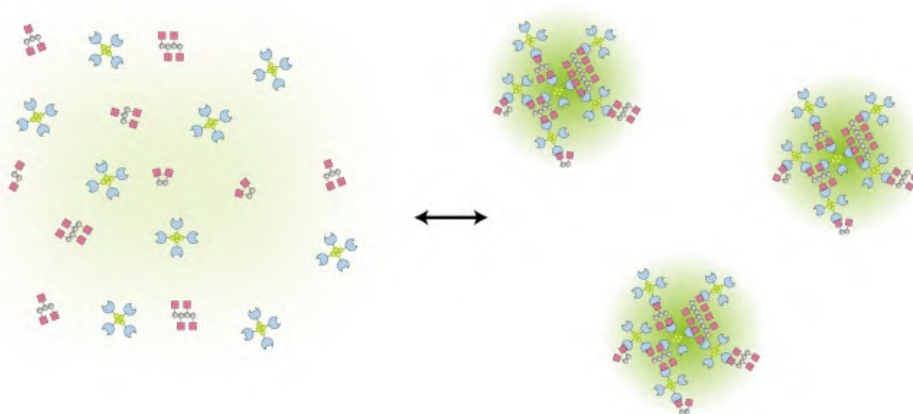


Figure 2 | Mechanism of action

2. Fluoppi : Ash-hAG [Bcl2-BAK]

This product contains two expression cassettes for detecting Bcl2-BAK interaction in living cells. One encodes a fusion protein Ash/Bcl2, and the other encodes humanized Azami-Green (hAG)/BAK. Partial sequences responsible for this interaction are used for this product. Co-transfection of DNA cassettes, Ash/Bcl2 and hAG/BAK, results in formation of cytoplasmic fluorescent puncta. After addition of Bcl2-BAK PPI inhibitors, the puncta disappeared within 5 hours, indicating the Bcl2-BAK complex was disrupted.

3. Product Components and Storage Condition

DNA cassettes	Amount:	Form
Ash/Bcl2	10 µg	Dry form
hAG/BAK	10 µg	Dry form

Reconstitute in 10-50 µL of sterilized distilled water before use.

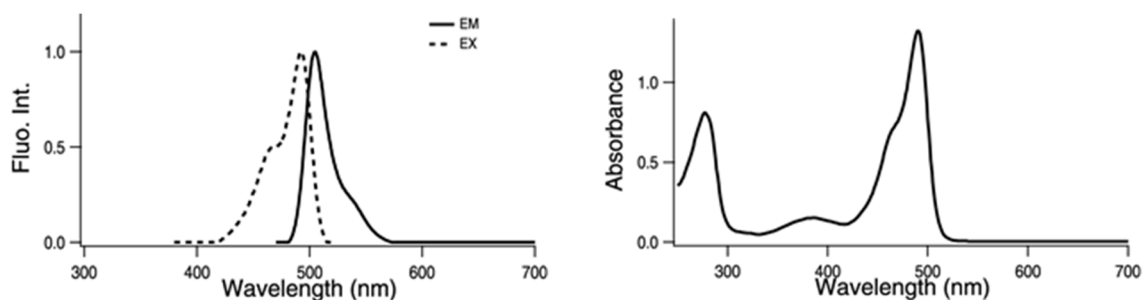
Storage condition: Store at -20°C. Reconstituted solution should be kept at -20°C.

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”

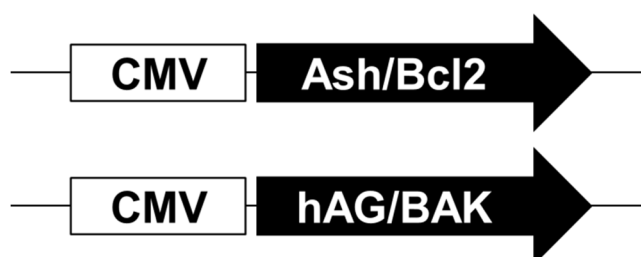
humanized Azami-Green (hAG), cloned from the stony coral (Azami-sango in Japanese), absorbs light maximally at 492 nm and emits green light at 505 nm. hAG forms tetramer and is featured by its fast maturation and highly photo and pH stable nature. The gene codon is optimized for mammalian cells.



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% CO₂ atmosphere. Cells were plated in collagen (KOKEN; code No. IAC-30) coated Lab-Tek Chambered Coverglass (Nunc; code No.155411) at 2x10⁴ 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 0.8 µL of FuGENE® HD Transfection Reagent (Promega; code No. E2311). 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.

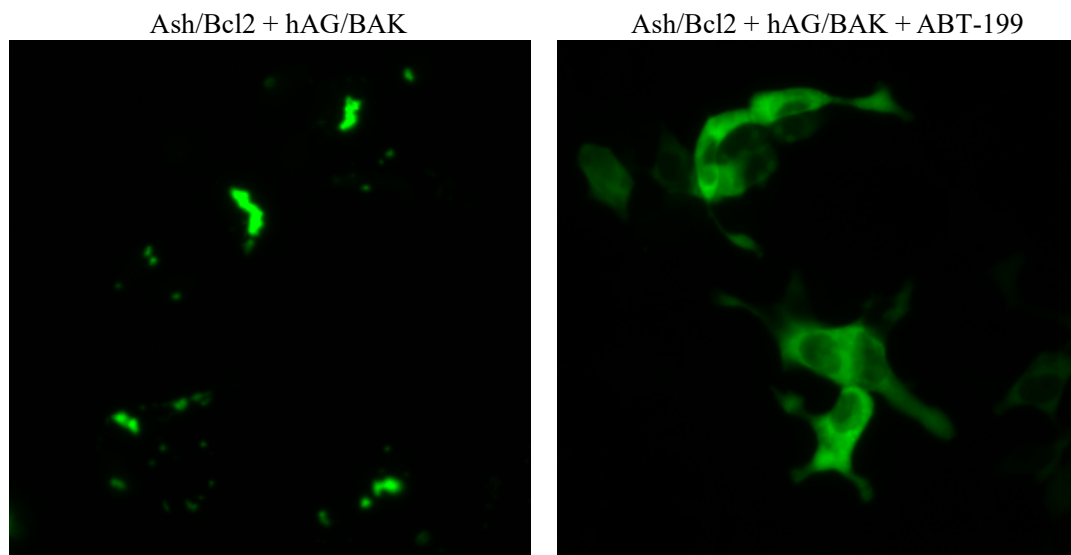


Figure 3 | HEK293 cells transiently expressing both Ash/Bcl2 and hAG/BAK were observed before (left) and 5 hours after addition of 10 µM ABT-199* (right). The interactions were observed as fluorescent puncta (left), and disruptions of the PPI by ABT-199 resulted in cytoplasmic diffused distribution of fluorescence (right).

* A Bcl2 selective inhibitor. (Souers, A.J., *et al.* 2013).

8. References

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

Souers, A.J., *et al.*, ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med.* 19, 202-208. (2013) [PMID: 23291630]

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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