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Fluorescent protein-protein interaction visualization

Fluoppi[†]

PPIs Detection Reagent : Fluoppi [Bcl2-BAK]

Code: AM-P1004

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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).

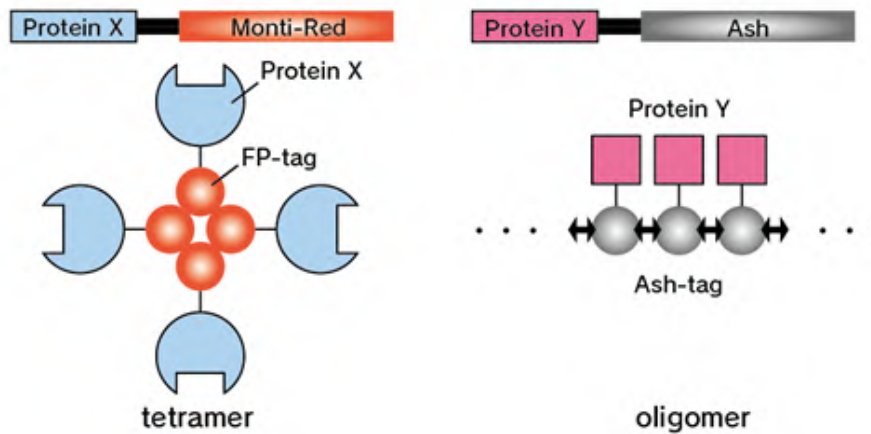


Figure 1 | Key components of Fluoppi technology

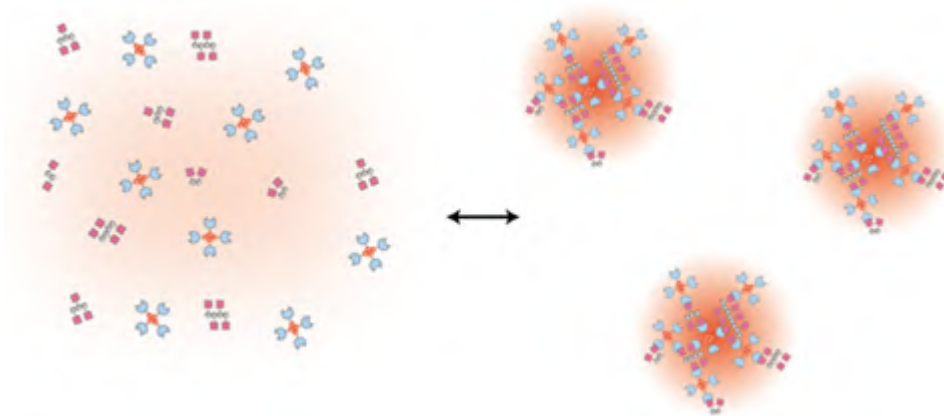


Figure 2 | Mechanism of action

2. Fluoppi : Ash-MR [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 Monti-Red (MR)/BAK. Partial sequences responsible for this interaction are used for this product. Co-transfection of DNA cassettes, Ash/Bcl2 and MR/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
MR/BAK	10 µg	Dry form

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

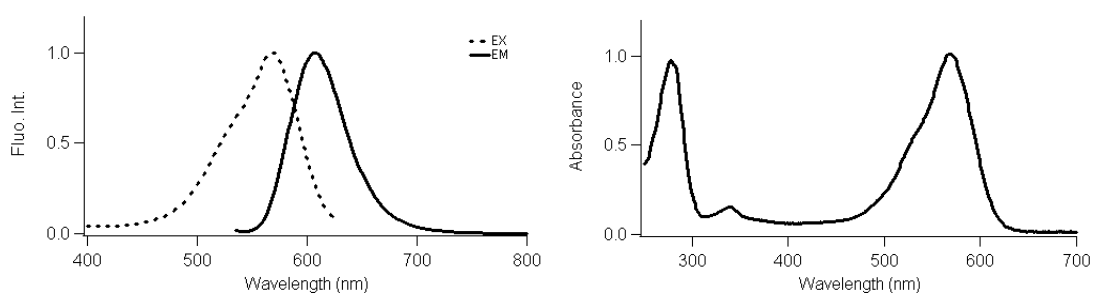
Storage condition: Store at -20°C. Reconstituted solution should be kept at -20°C.

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 “Monti-Red”

Monti-Red, a mutant fluorescent protein derived from Keima-Red which was originally cloned from the stony coral (*Montipora* sp.), forms tetramer and absorbs light maximally at 571 nm and emits red light at 607 nm. Fluorescent signal of Monti-Red can be detected by using filter sets for Texas Red or similar fluorescent dyes.



Fluorescent protein	Excitation/Emission maximum (nm)	Extinction coefficient ($M^{-1}cm^{-1}$)	Fluorescence quantum yield	pKa
Monti-Red	571/607	83,000 (571 nm)	0.3	5.5

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 MR fluorescence was performed by a 75-W Xenon lamp with a BP530-550 filter (Olympus). Emitted light was detected by an ORCA-Flash4.0 sCMOS camera (Hamamatsu Photonics) with a LP575 filter (Olympus) and a 570 nm dichroic mirror (Olympus). MetaMorph software (Molecular Devices) was used for data collections and analysis.

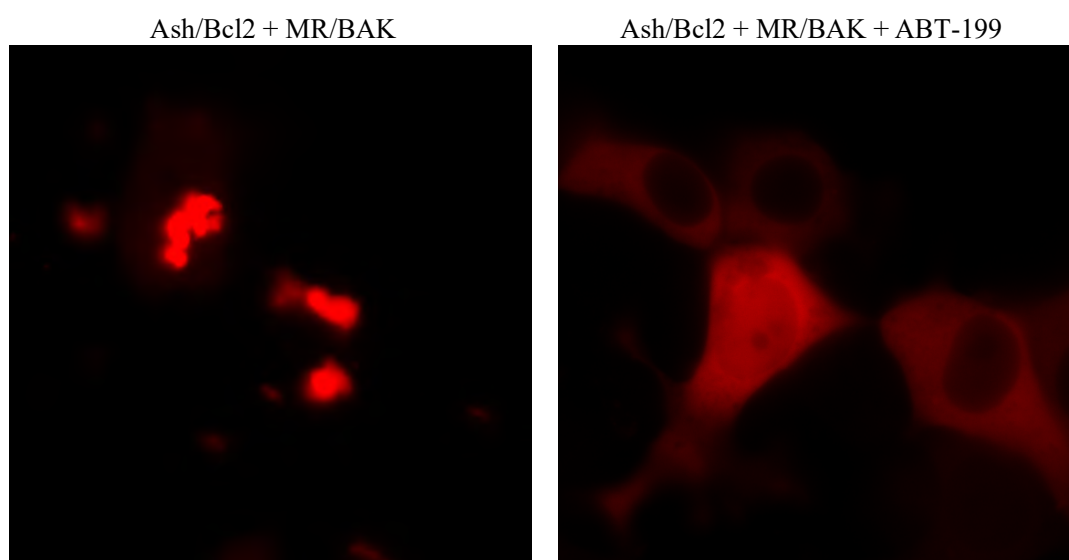


Figure 3 | HEK293 cells transiently expressing both Ash/Bcl2 and MR/BAK were observed before (left) and 5 hours after addition of 10 µM ABT-199* (right). The interactions were observed as fluorescent puncta (left), and disruptions of the PPI by ABT-199 resulted in cytoplasmic diffused distribution of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