Amalgaam

MBL

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Fluorescent protein-protein interaction visualization

Fluoppi

PPIs Detection Reagent : Fluoppi [Bcl2-BAK]

Code: AM-P1004

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).

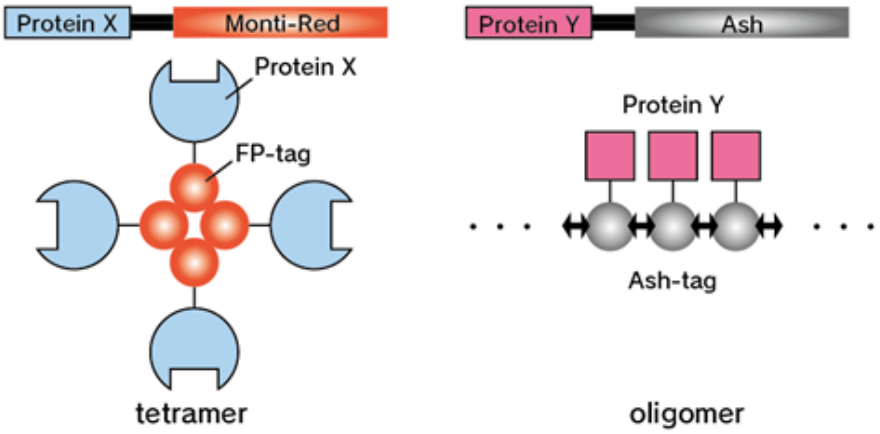


Figure 1 |Key components of Fluoppi technology

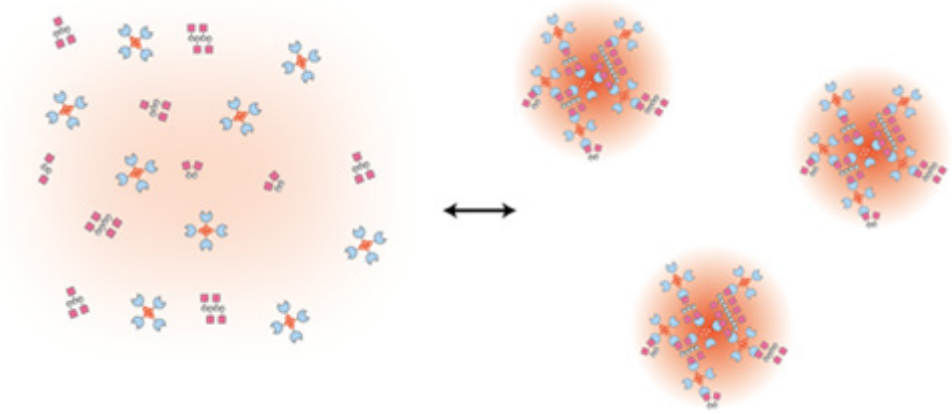


Figure 2 | Mechanism of action

2. Fluoppi : Ash-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

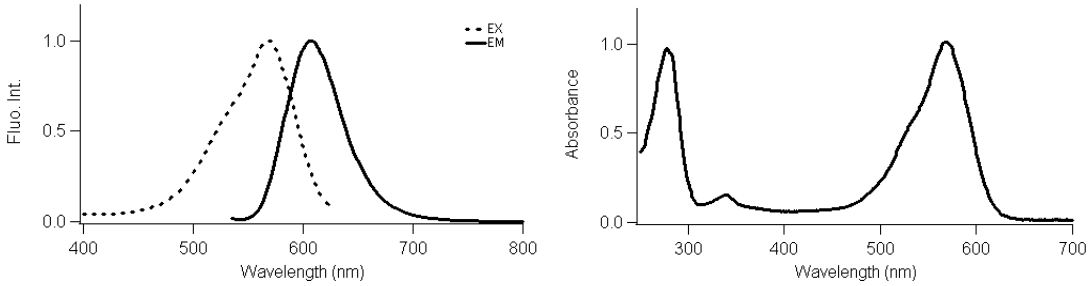
Reconstitute in 10-50 μL of sterilized distilled water before use.
Storage condition: Store at -20°C. Reconstituted solution should be kept at -20°C.

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% 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 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.

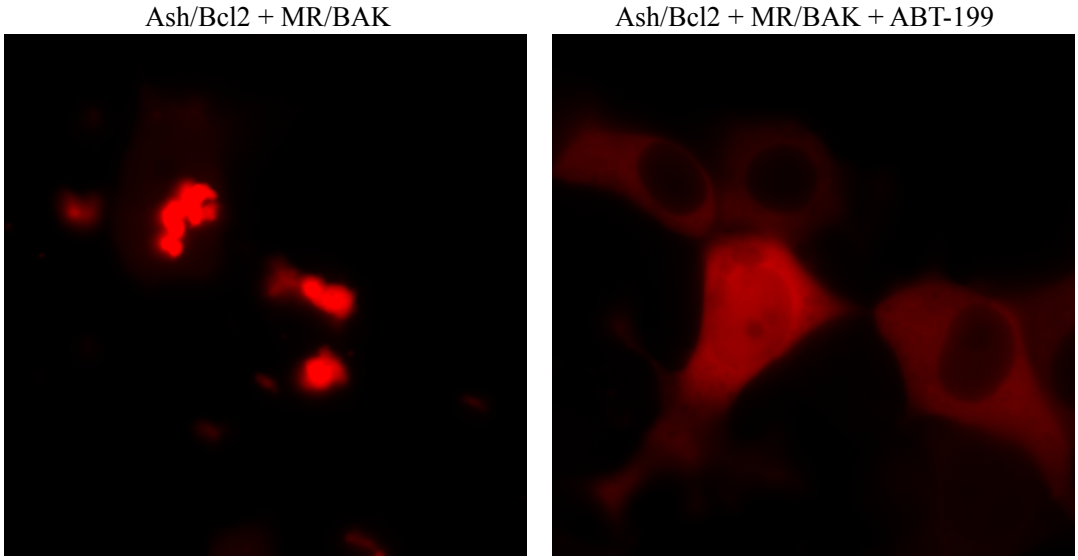


Figure 3 | HEK293 cells transiently expressing both Ash/Bcl2 and MR/BAK were observed before (left) and 5 hours after addition of 10 µM ABT-199* (right). The interactions were observed as fluorescent puncta (left), and disruptions of the PPI by ABT-199 resulted in cytoplasmic diffused distribution of 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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Printed Jan. 2018 /Version 1

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