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Fluorescent protein-protein interaction visualization

Fluoppi[†]

PPIs Detection Reagent : Fluoppi [Mcl1-BAX]

Code: AM-P1007

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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).

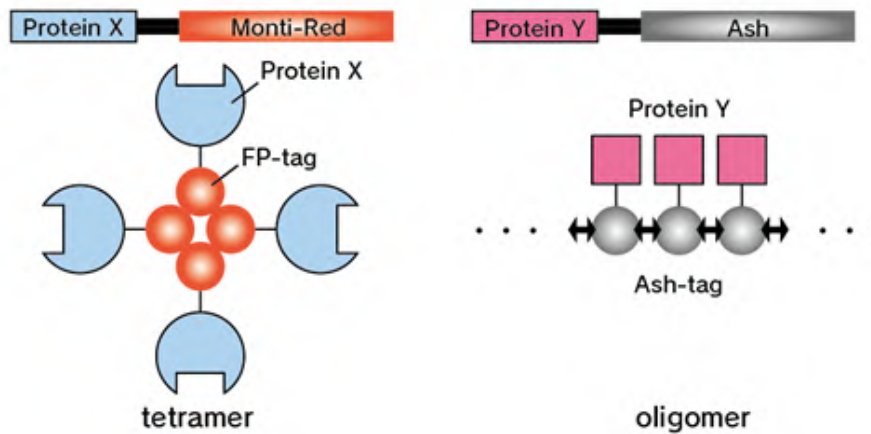


Figure 1 | Key components of Fluoppi technology

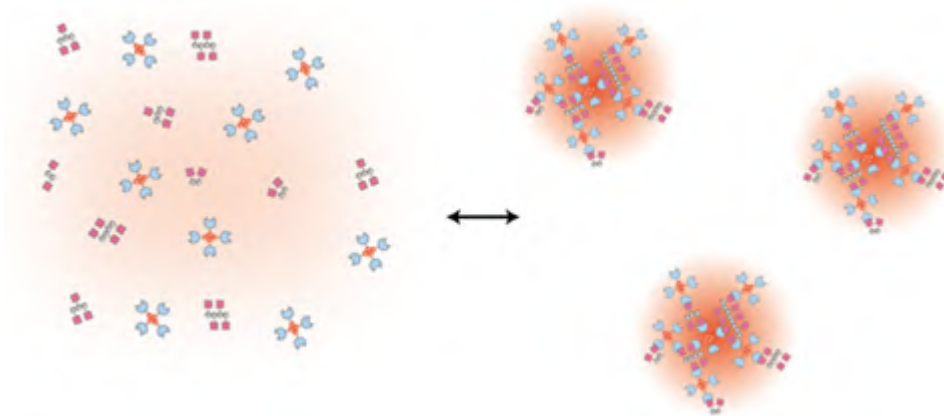


Figure 2 | Mechanism of action

2. Fluoppi : Ash-MR [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 Monti-Red (MR)/BAX. Partial sequences responsible for this interaction are used for this product. Co-transfection of DNA cassettes, Ash/Mcl1 and MR/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 |
| MR/BAX | 10 µg | Dry form |

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

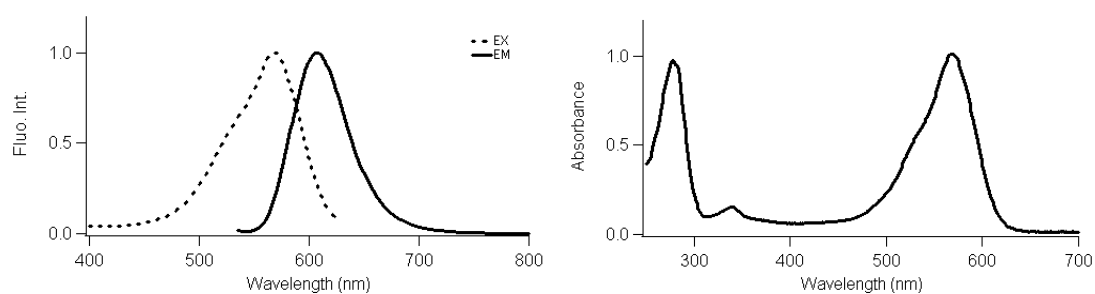
Storage condition: Store at -20°C. Reconstituted solution should be kept at -20°C.

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 “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.

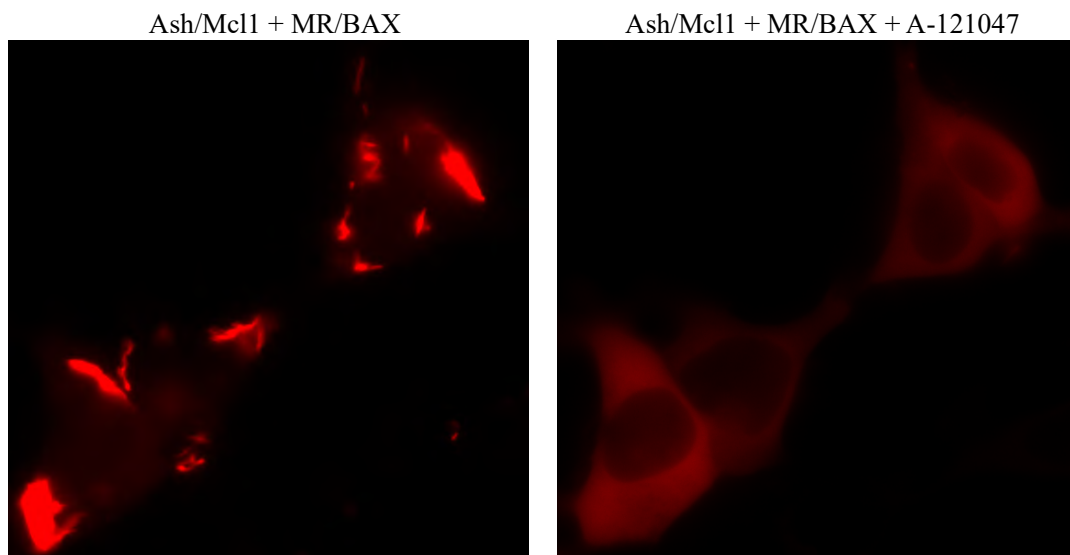


Figure 3 | HEK293 cells transiently expressing both Ash/Mcl1 and MR/BAX were observed before (left) and 30 minutes after addition of 25 µM A-121047* (right). The interactions were observed as fluorescent puncta (left), and disruptions of the PPI by A-121047 resulted in cytoplasmic diffused distribution 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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Printed September 1, 2025 /Version 2

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