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Fluorescent protein-protein interaction visualization

**Fluoppi**

PPIs Detection Reagent : Fluoppi [ BclxL-BAK ]

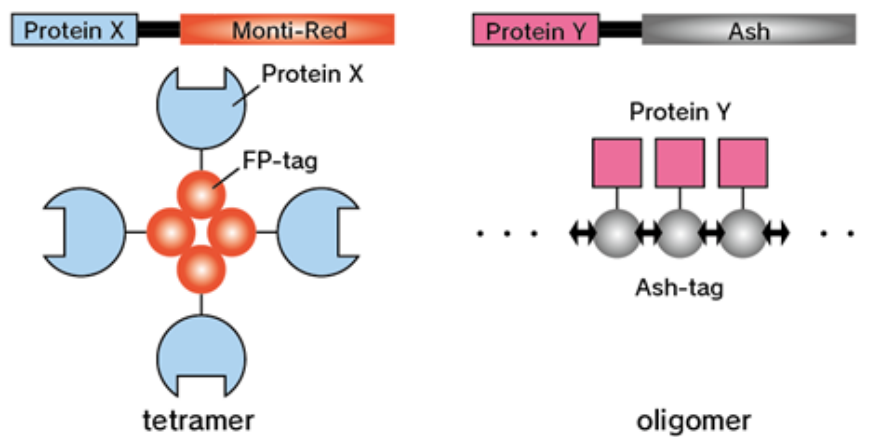
Code: AM-P1002

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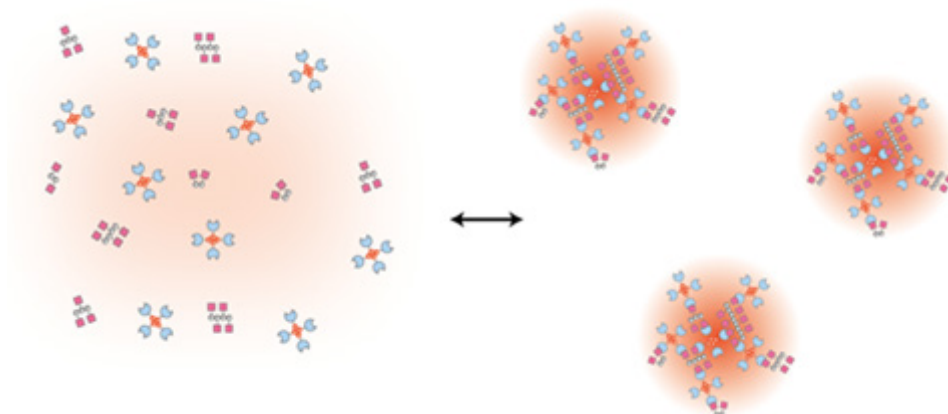
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## 1. Introduction

**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 [ BclxL-BAK ]

This product contains two expression cassettes for detecting BclxL-BAK interaction in living cells. One encodes a fusion protein Ash/BclxL, 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/BclxL and MR/BAK, results in formation of cytoplasmic fluorescent puncta. After addition of BclxL-BAK PPI inhibitors, the puncta disappeared within 4 hours, indicating the BclxL-BAK complex was disrupted.

## 3. Product Components and Storage Condition

DNA cassettes	Amount:	Form
Ash/BclxL	10 µg	Dry form
MR/BAK	10 µg	Dry form

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

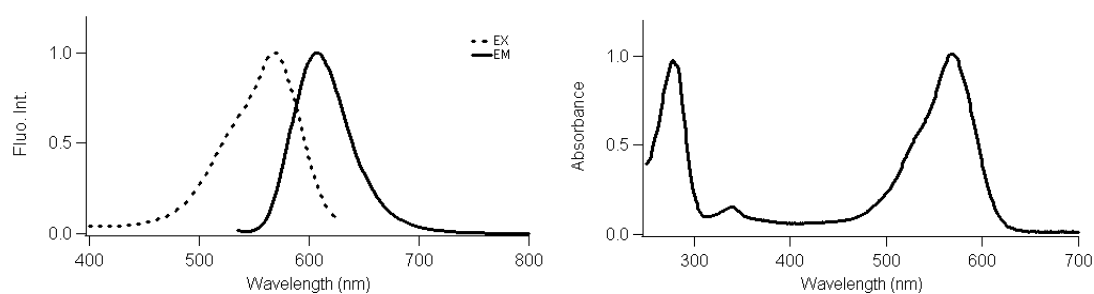
Storage condition: Store at -20°C. Reconstituted solution should be kept at -20°C.

## 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.



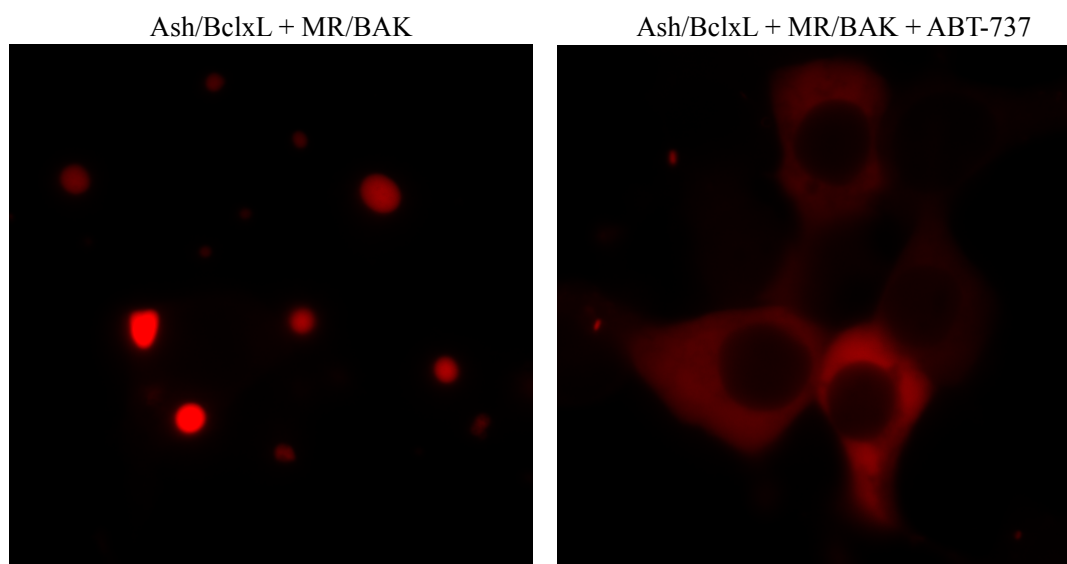
## 7. Example of Procedure

### [Transfection]

HEK293 cells were grown in DMEM (Sigma No. D5796) supplemented with 10% fetal bovine serum (FBS) and 1% Pen Strep (Gibco No. 15140-122) at 37°C in 5% CO<sub>2</sub> atmosphere. Cells were plated in collagen (KOKEN No. IAC-30) coated Lab-Tek Chambered Coverglass (Nunc No.155411) at  $2 \times 10^4$  cells per well with 200  $\mu$ L medium. After incubation for 16 hours, cells were transiently transfected with a pair of plasmid DNAs (both 200 ng) diluted in 10  $\mu$ L of Opti-MEM® (Gibco No. 31985-070) using FuGENE® HD Transfection Reagent (Promega, 0.8  $\mu$ L). 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/BclxL and MR/BAK were observed before (left) and 4 hours after addition of 25  $\mu$ M ABT-737\* (right). The interactions were observed as fluorescent puncta (left), and disruptions of the PPI by ABT-737 resulted in cytoplasmic diffused distribution of fluorescence (right).

\* An inhibitor of Bcl2, BclxL and Bclw (Oltersdorf, T., *et al.* 2005).

## 8. References

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

Oltersdorf, T., *et al.*, An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature. 435, 677-678. (2005) [PMID: 15902208]

## 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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**MBL** MEDICAL & BIOLOGICAL LABORATORIES CO.,LTD.

URL: <http://ruo.mbl.co.jp>

Email: [support@mbi.co.jp](mailto:support@mbi.co.jp)

Phone: +81-52-238-1904