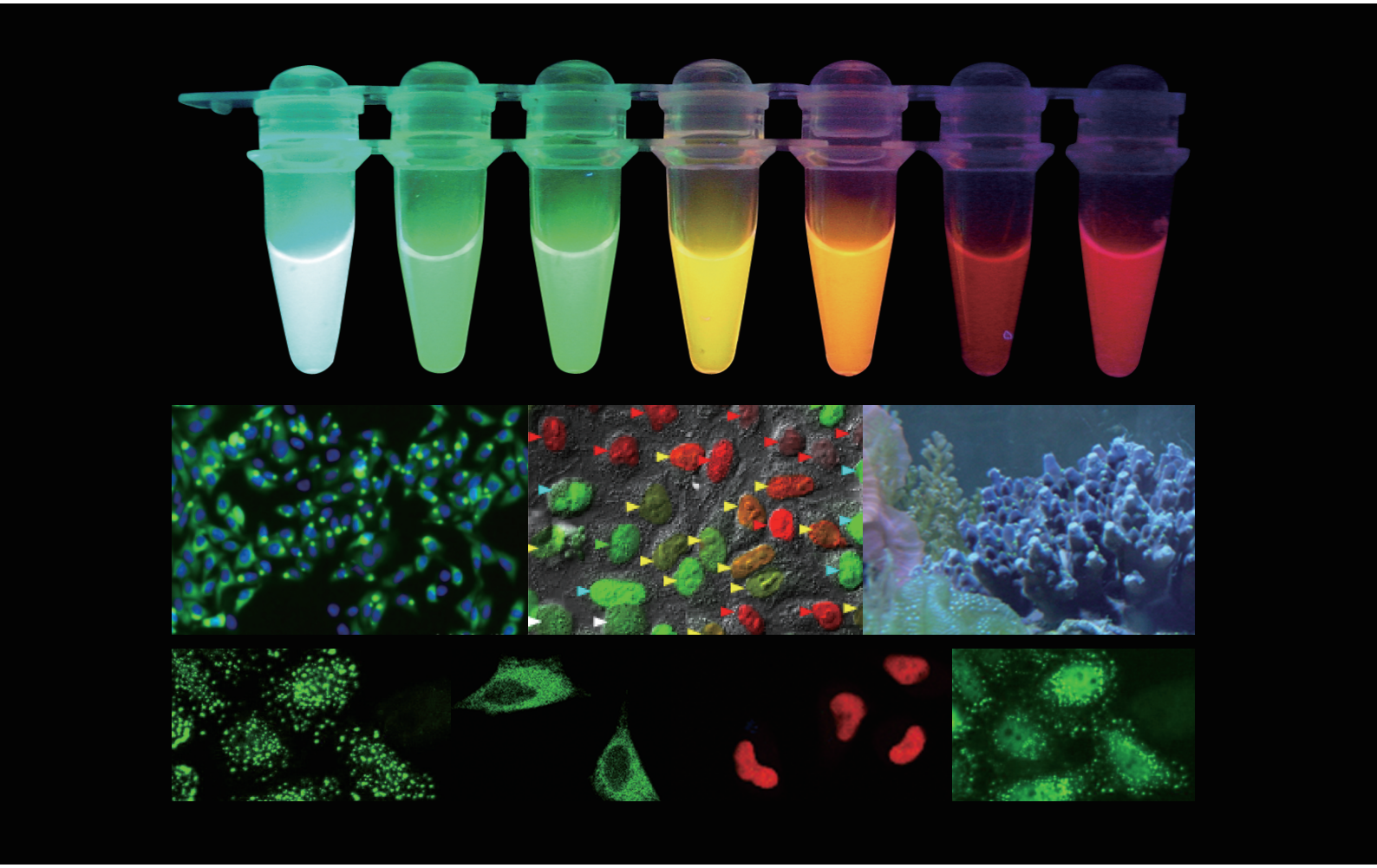
For Research Use Only

MBL
A JSR Life Sciences Company

Amalgaam
Fluorescent proteins



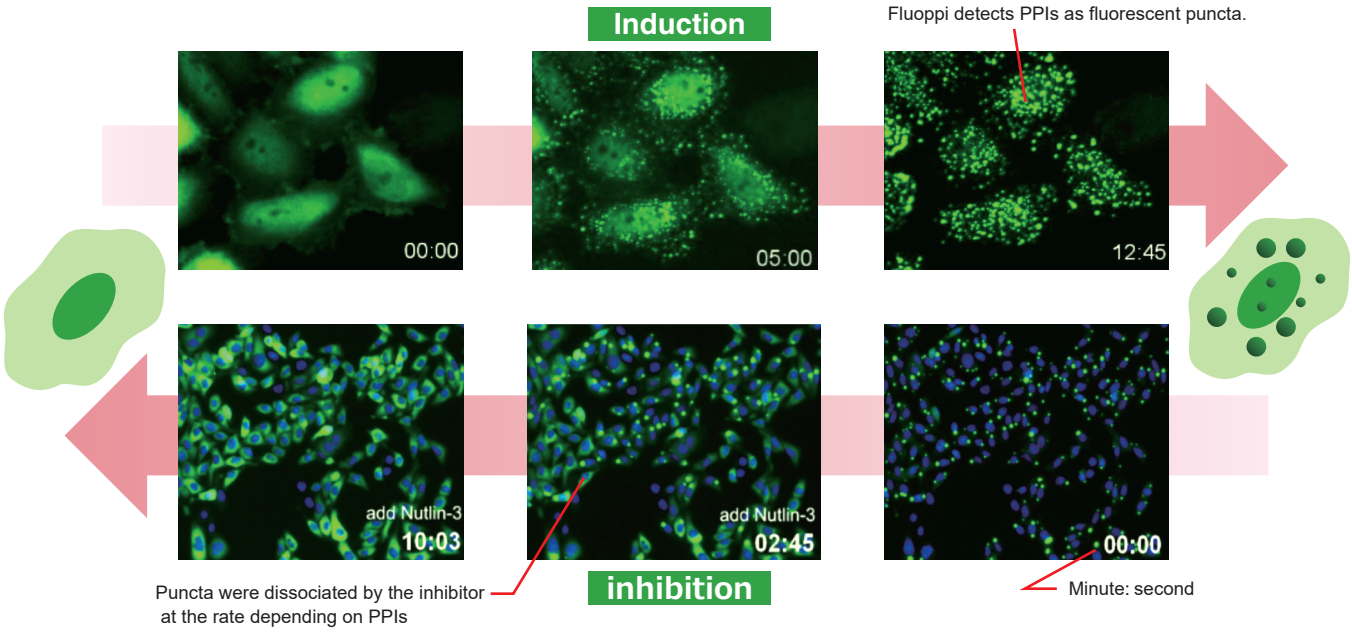
- Protein-Protein Interactions Detection System
- Advanced Fluorescent Indicator
- Antibodies

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

Fluoppi

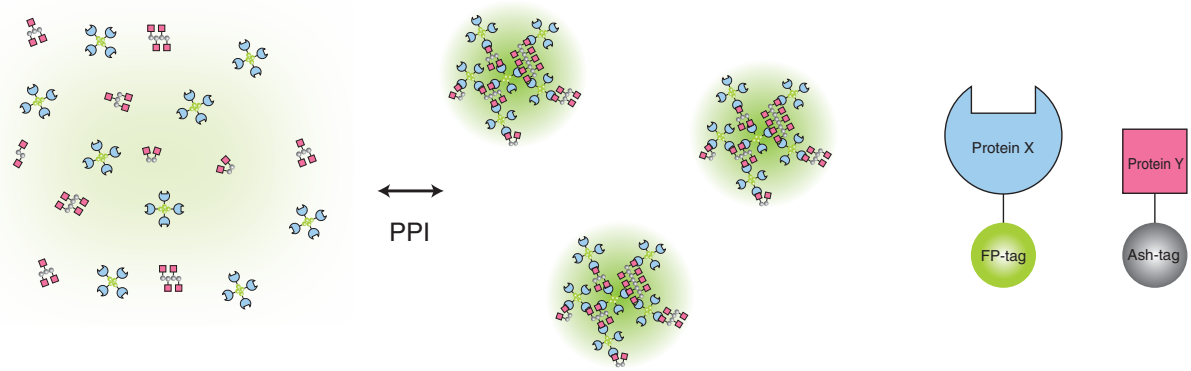
Novel technology for detecting PPIs

- **Visualize PPI as fluorescent "Puncta" in living cells**
- **"Reversible" puncta-formation**
- **Easy to construct an experimental system**
- **Ideal tool for drug screening**



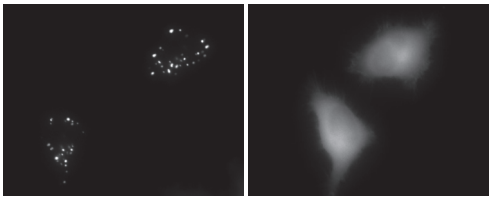
■ Mechanism of action

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. By way of example, 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, interaction between protein X and Y causes large lattice like complexes where the fluorescence by X-FP is concentrated and detectable as fluorescent puncta.

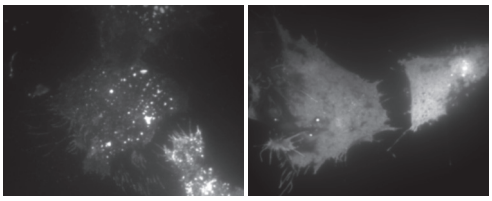


Localization

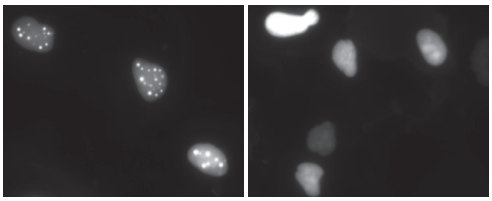
Because location of puncta is not restricted to specific site inside the cell, Fluoppi can detect PPIs at several subcellular localizations such as cytosol, nucleus, and juxtamembrane.
The left pictures represent puncta at several subcellular localizations, and the right pictures are negative controls which express hAG tagged protein and Ash-tag without fusing any proteins.
The images of juxtamembrane are taken by Total Internal Reflection Fluorescence Microscopy (TIRFM).



PPI in the cytosol | Negative control



PPI at juxtamembrane | Negative control

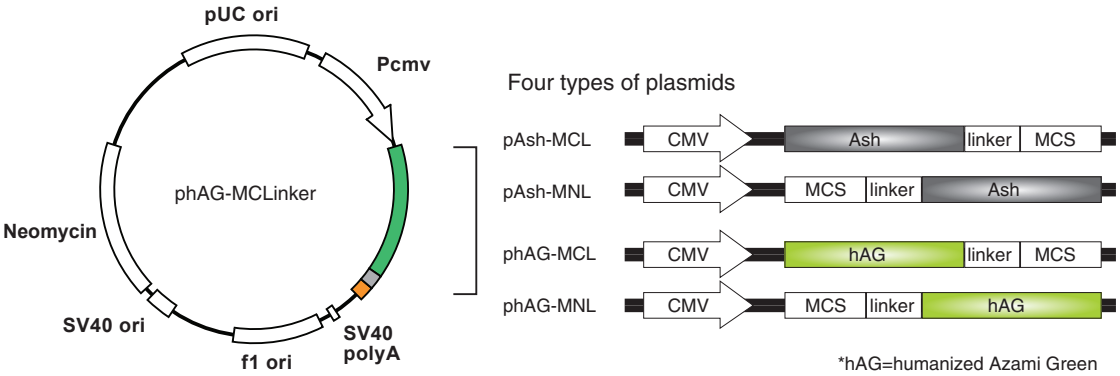


PPI in the nucleus | Negative control

Workflow

Fluoppi tags are able to work in both *N* and *C* terminal fusion. We have several plasmid vectors which include CMV promoter, Fluoppi tag, flexible peptide linker, Multiple Cloning Site (MCS) and Neomycin resistant gene.
At first, proteins X & Y of your interest are fused to FP-tag and Ash-tag respectively. We recommend to prepare all the eight possible constructs to identify the best workable combination.

Because fluorescent signal of Fluoppi is very high, conventional fluorescence microscopy can be used to image the cell.
If the proteins interact with each other upon expression, fluorescent puncta will be detected. Formation of puncta is reversible so that they can be dissociated and the fluorescent signal will spread over the cell by PPI inhibitors, and vice versa by PPI inducers.



*hAG=humanized Azami Green

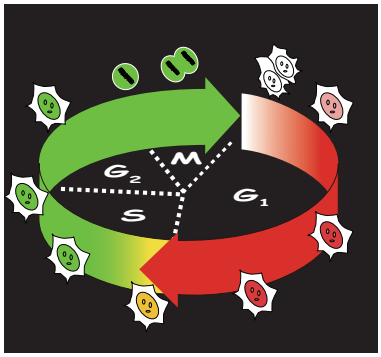
"Flexible"peptide linker (22 aa):
N term.- NSADG GGGSG GSGGS GGGST QG – *C* term.

Fluorescent proteins	Code No.	Products	Volume
Monti-Red (Red)	AM-8012M	Fluoppi Ver.2 : Ash-Red (Ash-MNL/MCL + Monti-Red-MNL/MCL)	10 μg each
	AM-VS0802M	Monti-Red for Fluoppi (pMonti-Red-MNL/MCL)	10 μg each
hAG (Green)	AM-8011M	Fluoppi Ver.2 : Ash-hAG (Ash-MNL/MCL + hAG-MNL/MCL)	10 μg each
	AM-8201M	Fluoppi : Ash-hAG [p53-MDM2]	10 μg each
	AM-8202M	Fluoppi : Ash-hAG [mTOR-FKBP12]	10 μg each
	AM-VS0801M	humanized Azami-Green for Fluoppi (phAG-MNL/MCL)	10 μg each

- This kit consists of four types of plasmids (pAsh-MCL, pAsh-MNL Ver.2, phAG-MCL, phAG-MNL)
- The use of these products requires a license from MBL Co., Ltd. MBL grants non-profit research organizations the right to use the product for non-commercial research purpose. For commercial entities a commercial license is required. For more information, please contact support@mbl.co.jp
- Fluoppi does not guarantee detection of all Protein-Protein Interactions.
- The fluorescent proteins used in product, hAzami-Green and Monti-Red, differ from each other in fluorescence and other properties.

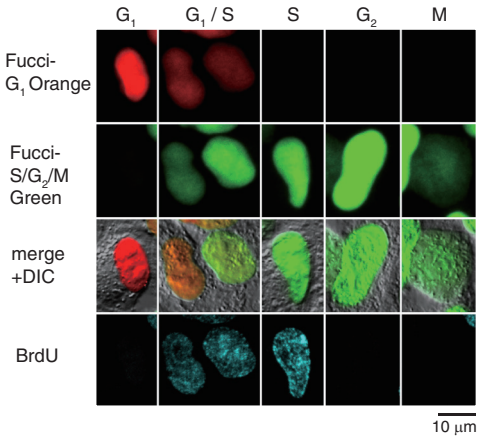
Fucci (Fluorescent Ubiquitination-based Cell Cycle Indicator)

- **Real-time visualization of cell-cycle progression**
- **Spatio-temporal imaging of cell cycle dynamics**



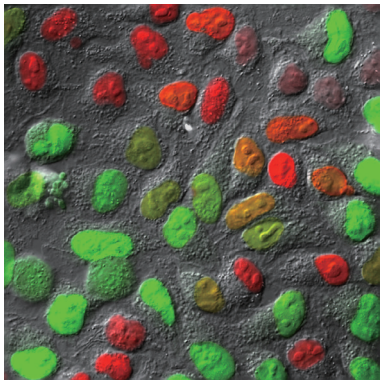
Fluorescent ubiquitination-based cell cycle indicator (Fucci) is a sophisticated technology which can visualize G_1 and/or S/G_2/ M phases of cell cycle in living cell. The mechanism of action of Fucci is based on ubiqitin-proteasome protein degradation system.
Fucci is a set of fluorescent probes: Fucci-G_1 Orange and Fucci-S/G_2/M Green. Fucci-G_1 Orange is a fusion protein of a fragment of human Cdt1 (amino acids 30-120) with the orange fluorescent mKO2 (monomeric Kusabira-Orange 2) that indicates the G_1 phase. Fucci-S/G_2/M Green is a fusion protein of a fragment of human Geminin (amino acids 1-110) with the green fluorescent protein mAG1 (monomeric Azami-Green 1) that visualizes S, G_2 and M phases.

Fluorescent images of Fucci cells



Each cell cycle of the G_1, G_1/S , S, G_2, and M phases can be determined by the combination of Fucci-G_1 Orange, Fucci-S/G_2/M Green, and an antibody against PCNA. G_1 phase is indecated by orange. Both orange and green were observed in the G_1/S phase. Additional immunostaining color by PCNA was observed at the initiation of the S phase. Cells with pure green fluorescence were either in the S or G_2 phase and were distinguished by immunostaining of the S phase. The rest of the cells were classified into the M phase.

Fucci stable transfectant (HeLa cells)



This product is licensed from RIKEN and the Tokyo Metropolitan Institute of Medical Science.

Volume: 20 μg

Code No.	Products
AM-V9001M	pFucci-G_1 Orange (Cloning vector)
AM-V9003M	pFucci-G_1 Orange (Expression vector)
AM-V9010M	pFucci-S/G_2/M Green-Hyg (Expression vector)
AM-V9014M	pFucci-S/G_2/M Green (Cloning vector)
AM-V9016M	pFucci-S/G_2/M Green (Expression vector)
AM-V9030M	pFucci-S/G_2/M Green(N+C)-Hyg (Expression vector)
AM-V9034M	pFucci-S/G_2/M Green(N+C) (Cloning vector)

The use of these products requires a license from MBL Co., Ltd. MBL grants non-profit research organizations the right to use the product for non-commercial research purpose. For commercial entities a commercial license is required. For more information, please contact support@mbl.co.jp

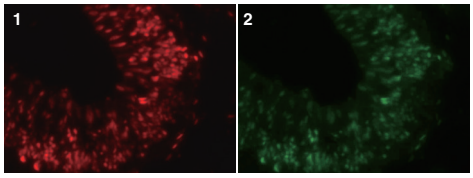
Anti-Fluorecsent Protein Antibodies

Code no.	Products	Clone	Volume	Applications	Western Blot cross reactivity
M102-3M	Anti-monomeric Azami-Green1 mAb	2F11	100 μg/100 μL	WB	mAG1
PM052M	Anti-monomeric Azami-Green1 pAb	Polyclonal	100 μL	WB, IP, IC, IH	mAG1
PM011M	Anti-Azami-Green pAb	Polyclonal	100 μL	WB	AG, mAG1
M106-3M	Anti-Kaede mAb	2F4	100 μg/100 μL	IP	
M125-3M	Anti-Kaede mAb	3B1	100 μg/100 μL	WB	
PM012M	Anti-Kaede pAb	Polyclonal	100 μL	WB	
M126-3M	Anti-monomeric Keima-Red mAb	2F7	100 μg/100 μL	WB	mKeima-Red
M128-3M	Anti-Kikume Green-Red mAb	5B3	100 μg/100 μL	WB	KikGR, mKikGR
M182-3M	Anti-Keima-Red mAb	1C3	100 μg/100 μL	WB	
M104-3M	Anti-monomeric Kusabira-Orange1 mAb	1H7	100 μg/100 μL	WB	mKO1, mKO2, mKG, mKG-O, mKOkappa
M168-3M	Anti-monomeric Kusabira-Orange2 mAb	3B3	100 μg/100 μL	WB, IP, IC, IH	mKO2, mKG, mKG-O, mKOkappa
PM051M	Anti-monomeric Kusabira-Orange2 pAb	Polyclonal	100 μL	WB, IP, IC, IH	KO1,mKO1,mKO2,mKG,mKG-O,mKOkappa
M116-3M	Anti-Midoriishi-Cyan mAb	2C1	100 μg/100 μL	IP	
M130-3M	Anti-Midoriishi-Cyan mAb	5B7	100 μg/100 μL	WB	MiCy, mMiCy
M148-3M	Anti-monomeric Kusabira-Green N-terminal Fragment mAb	1E6	100 μg/100 μL	WB	mKO1, mKO2, mKG
M149-3M	Anti-monomeric Kusabira-Green C-terminal Fragment mAb	21B10	100 μg/100 μL	WB	mKO2, mKG
M223-3	Anti-Ash-tag mAb	FLP1C15-2	100 μg/100 μL	WB	Ash-MCL, Ash-MNL Ver.2

WB: Western blotting, IP: Immunoprecipitation, IC: Immunocytochemistry, IH: Immunohistochemistry

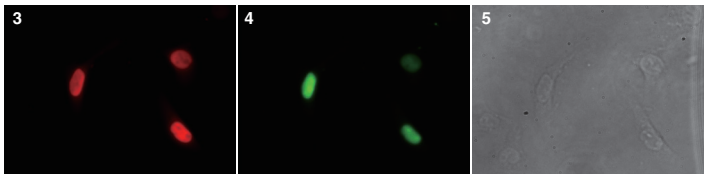
Anti-monomeric Azami-Green1 pAb (Code No. PM052M)

Immunohistochemistry



Immunohistochemical detection of mAG1 on frozen section of B6.Cg-Tg (Fucci) 504Bsi mouse embryonic brain (E13) with PM052M (1) and Fucci-S/G_2/M Green own fluorescence (2).

Immunocytochemistry

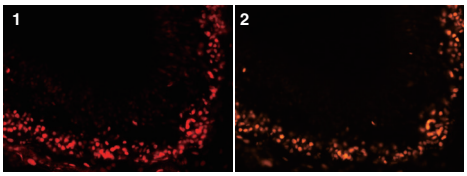


Immunocytochemical detection of mAG1 in Fucci-S/G_2/M Green transfected HeLa cells with PM052M.

3: Anti-mAG1 (PM052M)
4: Fucci-S/G_2/M Green
5: Transmission light

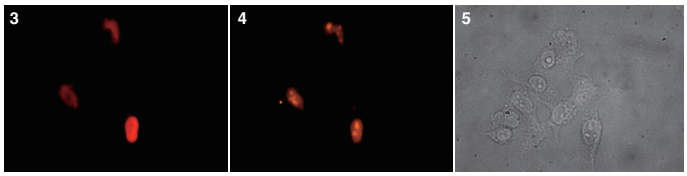
Anti-monomeric Kusabira-Orange2 mAb (Code No. M168-3M)

Immunohistochemistry



Immunohistochemical detection of mKO2 on frozen section of B6. Cg-Tg (Fucci) 596Bsi mouse embryonic brain (E12) with M168-3M (1) and Fucci-G_1 Orange own fluorescence (2).

Immunocytochemistry



Immunocytochemical detection of mKO2 in Fucci-G_1 Orange transfected HeLa cells with M168-3M.

3: Anti-mKO2 (M168-3M)
4: Fucci-G_1 Orange
5: Transmission light

For research use only. Not for use in diagnostic or therapeutic procedures.
The information is as of April 2024. Please contact us for the latest information. Please read the data sheets carefully before use.

2024.04 156001-24041000

MEDICAL & BIOLOGICAL
LABORATORIES CO., LTD.
A JSR Life Sciences Company

SUMITOMO FUDOSAN SHIBADAIMON NICHOME BLDG.
2-11-8 Shibadaimon, Minato-ku, Tokyo 105-0012 Japan
E-mail: support@mbl.co.jp
URL: https://www.mblbio.com/bio/g/