For Research Use Only.
Not for use in diagnostic procedures.

Amalgaam MBL

Fluorescent Protein Expression vector set

humanized Azami-Green for Fluoppi (phAG-MNL/MCL)

Code No.

AM-VS0801M

Introduction:

This product consists of two types (N or C-terminus fusion) of tetramer green fluorescent protein expression vectors for Fluoppi analysis. phAG-MNL and phAG-MCL expresses a protein of interest fused to the N-terminus and the C-terminus of humanized Azami Green (hAG), respectively. To reduce the interference between a protein of interest and fluorescent protein, these vectors are designed to insert more than 22 amino acids length flexible linker peptide between them. For the details of the vectors and property of hAG, please refer to the information below.

Note: Because this product does not contain the Ash-tag expression vectors, basic Fluoppi kit (e.g. Fluoppi : Ash-Red, code no. AM-8002M) is necessary for Fluoppi analysis.

Product components:

Plasmids	Vial color	Form
phAG-MNL	Green	10 µg: Dry form
phAG-MCL	Green	10 µg: Dry form

* Reconstitution in 10-50 µL of sterilized distilled water.

Storage condition:

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

Intended use:

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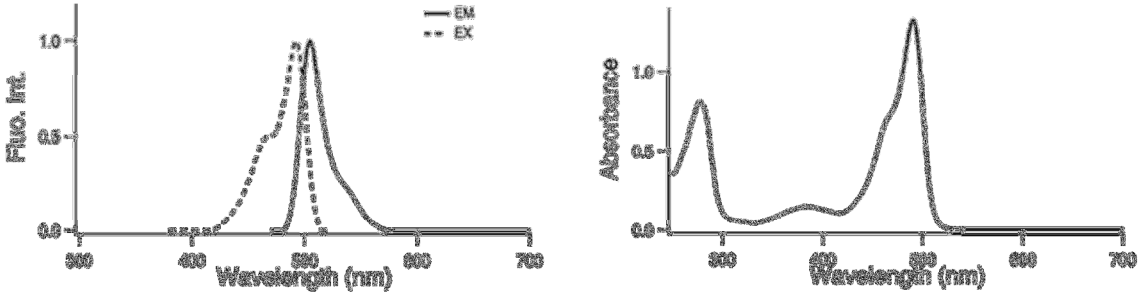
Properties of Fluorescent protein “hAG”:

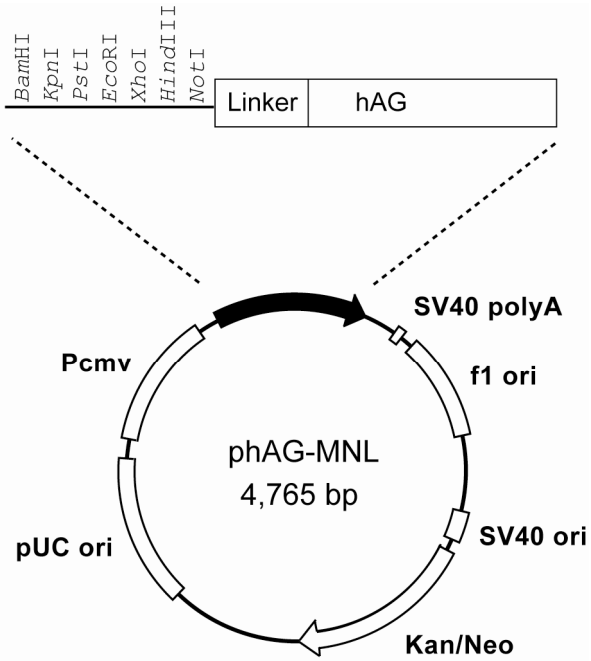
humanized Azami-Green (hAG), cloned from the stony coral (azami-sango in Japanese), absorbs light maximally at 492 nm and emits green light at 505 nm. hAG forms tetramer and is featured by its fast maturation and high stability nature. The gene codon is optimized for mammalian cells.



Fluorescent protein	Excitation/Emission maximum (nm)	Extinction coefficient ($M^{-1}cm^{-1}$)	Fluorescence quantum yield	pKa
hAG	492/505	72,300 (492 nm)	0.67	<5.0

Sequence information of the two plasmids can be downloaded from our website.
http://ruo.mbl.co.jp/product/flprotein/fluoppi.html

Plasmid map:



SEQUENCE LANDMARKS:
hAG gene: bases 145-822
peptide linker: bases 73-144
CMV promoter: bases 4175-4747
SV40 polyA: bases 985-1019
Kanamycin/Neomycin resistance gene: bases 2062-2853
pUC origin: bases 3441-4084
f1 origin: bases 1082-1537
SV40 origin: bases 1878-2013

Linker, MCS and the annealing site of recommended sequence primer (MN-Forward)

MN-Forward

5 '- ACA ACT CCG CCC CAT TGA CGC AAA TGG GCG GTA GGC GTG TAC GGT GGG AGG TCT ATA TAA GCA GAG

MCS

BamHI *KpnI* *PstI* *EcoRI*

CTG GTT TAG TGA ACC GTC AGA TCC GCT AGC ATT GGA TCC TCA GGT ACC GGA ACT GCA GCA GAG AAT
G S S G T G T A A E N

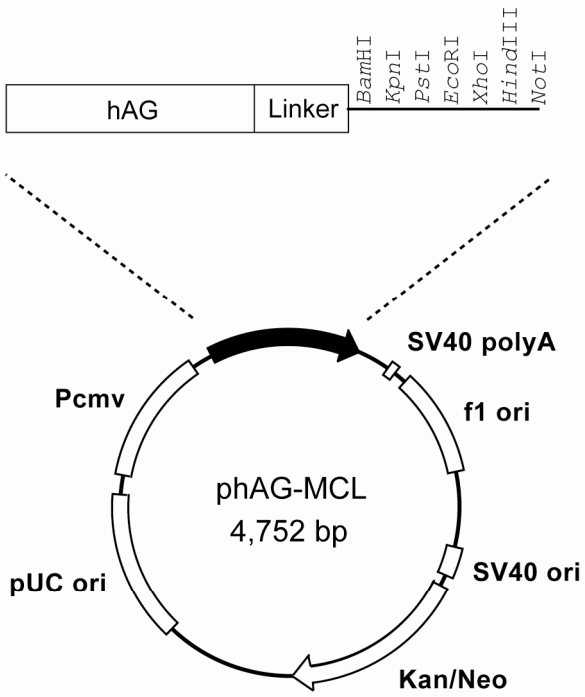
Linker

XhoI *HindIII* *NotI*

TCG GGA AAC TCG AGA ACA AAG CTT GGA TCA GCG GCC GCC AAT TCC GCT GAC GGC GGC GGA GGA TCG
S G N S R T K L G S A A A N S A D G G G G S

hAG

GGT GGT AGT GGT GGT TCA GGA GGA GGA TCG ACC CAA GGA ACC GGT - 3'
G G S G G S G G G S T Q G T G



SEQUENCE LANDMARKS:
hAG gene: bases 1-678
peptide linker: bases 679-750
CMV promoter: bases 4156-4728
SV40 polyA: bases 966-1000
Kanamycin/Neomycin resistance gene: bases 2043-2834
pUC origin: bases 3422-4065
f1 origin: bases 1063-1518
SV40 origin: bases 1859-1994

Linker, MCS and the annealing site of recommended sequence primer (MC-Reverse)

Linker

5'- ACC GGT AAT TCC GCT GAC GGC GGC GGA GGA TCG GGT GGT AGT GGT GGT TCA GGA GGA GGA TCG ACC
T G N S A D G G G G S G G S G G S G G G S T

MCS

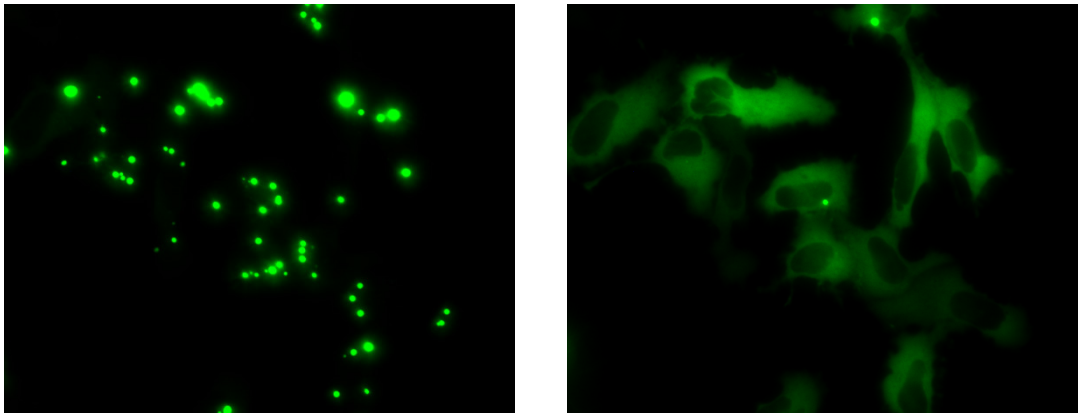
BamHI *KpnI* *PstI* *EcoRI* *XhoI* *HindIII*
CAA GGA GGA TCC TCA GGT ACC GGA ACT GCA GCA GAG AAT TCG GGA AAC TCG AGA ACA AAG CTT GAA
Q G G S S G T G T A A E N S G N S R T K L E

NotI
TAA GCG GCC GCG ACT CTA GAT CAT AAT CAG CCA TAC CAC ATT TGT AGA GGT TTT ACT TGC TTT AAA
*

MC-Reverse
AAA CCT CCC ACA CCT CCC - 3'

: stop codon

Example of Fluoppi assay:



HeLa-S3 cells transiently expressing both Ash/p53 and hAG/MDM2 were observed at 0 minute (left) and 15 minutes (right) after addition of 10 μM Nutlin-3. The interactions were observed as fluorescent foci (left), and disruptions of the PPI by Nutlin-3 resulted in a cytoplasmic evenly distribution of fluorescence (right).

Reference:

Karasawa, S., *et al., J. Biol. Chem.* **278**, 34167-34171 (2003)

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)