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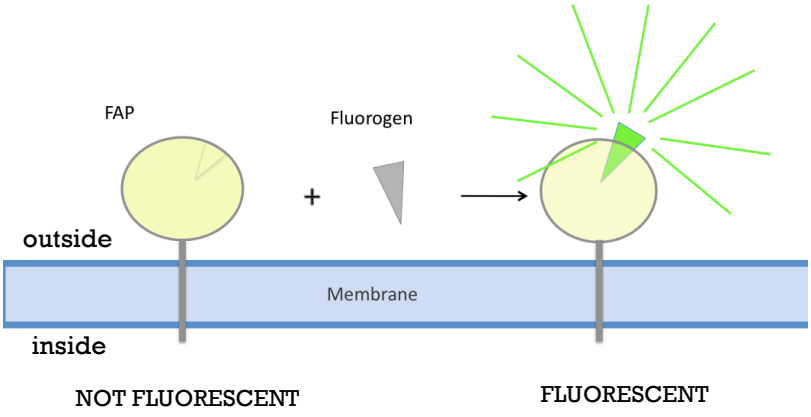
FAP-Tagging Kit for Live-Cell Assays

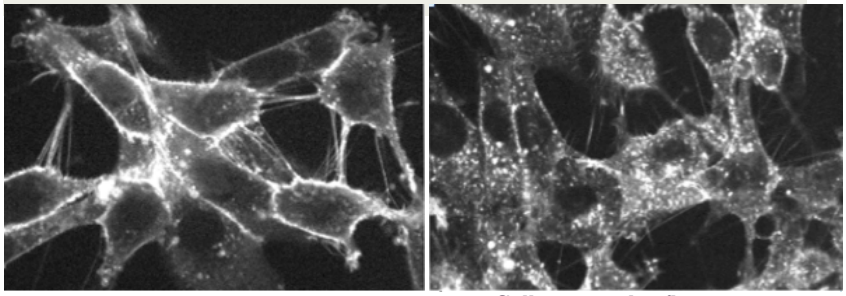


Features and Benefits

FAP-tags® are a new class of small genetically encoded reporters that exhibit fluorescence only in the presence of micromolar concentrations of particular nontoxic soluble fluorogens. FAP-tags® allow the user to turn the fluorescent signal on and off by adding or removing fluorogen, or to change the signal wavelength by substituting one fluorogen for another.

When used in conjunction with membrane-impermeable fluorogens, FAP-tags® have proved particularly useful in live-cell assays that monitor the translocation of membrane proteins to or from the cell surface.

- Signal is dependent on the presence of fluorogen; no background unless fluorogen is present.
- Immediate appearance of signal upon addition of fluorogen to live or fixed cells.
- Multiple colors available; color is dependent on FAP-fluorogen pair.
- Signal intensity comparable to fluorescent proteins.
- Specific detection and quantification of proteins at the cell surface through the use of membrane impermeant fluorogens.
- Small size (~25 kDa).
- Nontoxic.



Resting cells exposed to fluorogen

Cells exposed to fluorogen plus CCR5 agonist

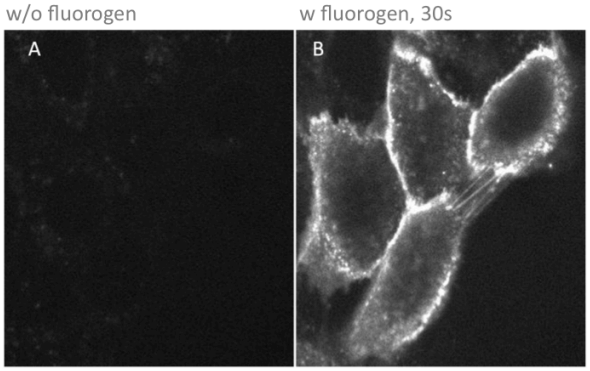
NIH 3T3 cells expressing FAP-tagged CCR5

OVERVIEW

Fluorogen-Activating Proteins (FAPs) are proteins that noncovalently bind fluorogenic dyes (fluorogens) with submicromolar affinity, leading to the acquisition of strong fluorescence [1]. Neither the FAP nor the fluorogen is fluorescent by itself. The FAP-fluorogen complex is called a fluoromodule.

Cells that express membrane-anchored FAP tags are readily generated using SpectraGenetics' pMFAP vectors. Signal appears immediately upon addition of fluorogen to the medium. Signal can be detected and quantified by fluorescence microscopy, fluorimetry or flow cytometry[2,3]

[1] Szent-Gyorgyi et al, Nat Biotechnol. 26:235-40, 2008
[2] Holleran et al, CytometryA, 2010
[3] Fisher et al., J. Biomol. Screening, 2010



Each kit contains the following components:

- pMFAP vector supplied as *E. coli* stab.
- Lyophilized membrane-impermeant fluorogen sufficient for 20 experiments with a media volume of 1 ml each, or 100 experiments with a volume of 200 µL each.
- Product Manual.

Storage

Products are shipped at room temperature. E. coli stabs may be stored for up to two weeks at 4°C. Lyophilized fluorogens may be stored for up to a year at room temperature.

Publications, Protocols, and Fluoromodule Properties

Protocols for cell culture and FAP detection may be found in the following publications:

1. Szent-Gyorgyi et al., Nature Biotechnology 26:235-240, 2008. pdf
2. Holleran et al., Cytometry A 77:776-782, 2010. pdf
3. Fisher et al., Journal of Biomolecular Screening 15:703-709, 2010. pdf
4. Holleran et al., Molecular Medicine 18, 2012. pdf

For standard (not confocal) fluorescence microscopy, we recommend that the medium be changed to PBS prior to addition of fluorogen.

The table below shows the correspondence between the SpectraGenetics product names and the FAP and fluorogen names used in the publications list.

Spectra Product Name	Alternative Names
FAPα1	HL4-MG, MG13
FAPα2	L5-MG dimer, dNP138
FAPβ1	HL1.0.1-TO1, AMII.2
FAPβ2	HL1-TO1 (scFv1)
αRED-np1	MG-11p
αRED-p1	MG-ester
βGREEN-np1	TO1-2p

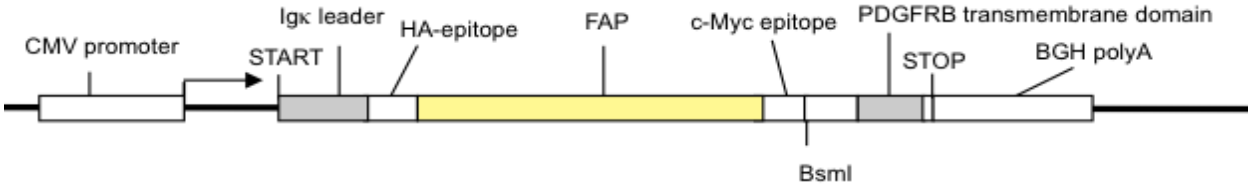
Fluoromodule properties:

FAP	Fluorogen*	Excitation Max	Emission Max	Kd	Recommended fluorogen working concentration
FAPα1	αRED-np	631 nm	650 nm	3.0 nM	100 nM
	αRED-p	629 nm	649 nm	3.2 nM	100 nM
FAPα2	αRED-np	631 nm	650 nm	< 1 nM	100 nM
	αRED-p	640 nm	668 nm	< 1 nM	100 nM
FAPβ1	β1-GREEN-np	509 nm	530 nm	3.1 nM	100 nM
FAPβ2	β2-GREEN-np	510 nm	527 nm	360 nM	500 nM

*p: membrane permeant fluorogen
*np: membrane-impermeant fluorogen

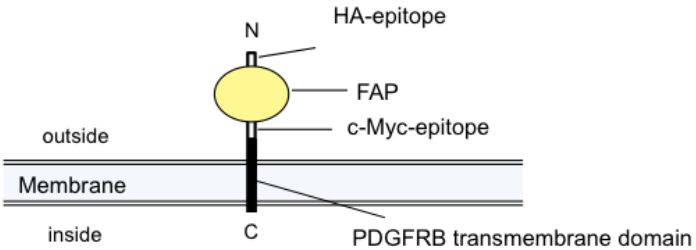
Cell surface signal may be visualized as early as one minute after addition of membrane-impermeant fluorogen. Internal signal may be visualized as early as five minutes after addition of membrane-permeable fluorogen.

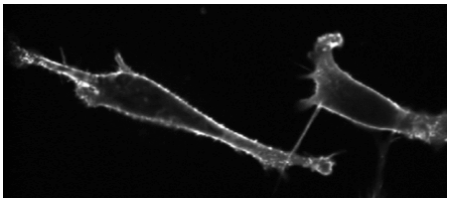
Organization and Topology of Proteins Expressed from pMFAP



The structure of the FAP expression unit in the pMFAP vector

FAP expression at the plasma membrane: Open reading frames (ORF's) encoding proteins of interest may be cloned into the BsmI site of the pMFAP vector.

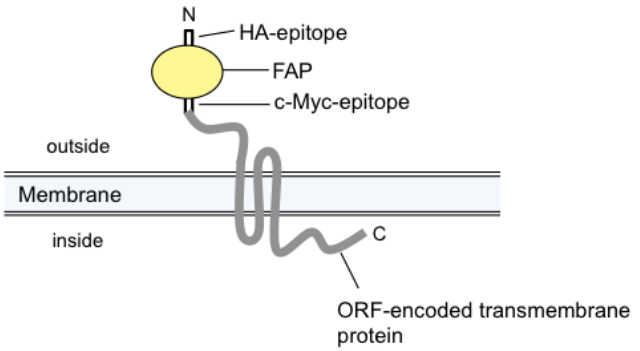




NIH 3T3 cells expressing FAP at surface

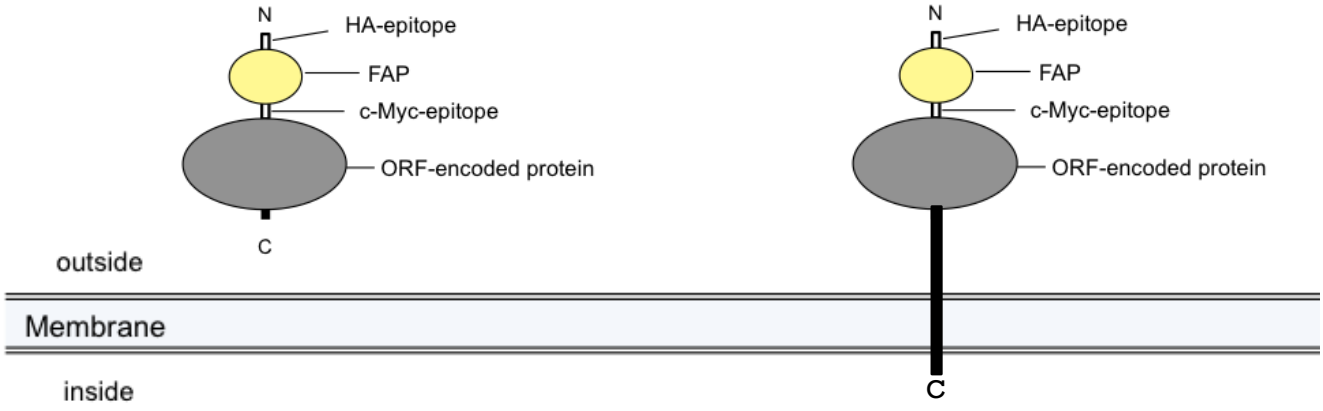
FAP vector alone

Cells transfected with a pMFAP1 vector with no insert express membrane-anchored FAPs on their surface.



FAP-tagged fusion proteins

Membrane protein with stop codon: If the ORF encodes a membrane protein with an extracellular N-terminus, and if the ORF ends with a stop codon, a fusion protein with an N-terminal FAP-tag is expressed at the cell surface.



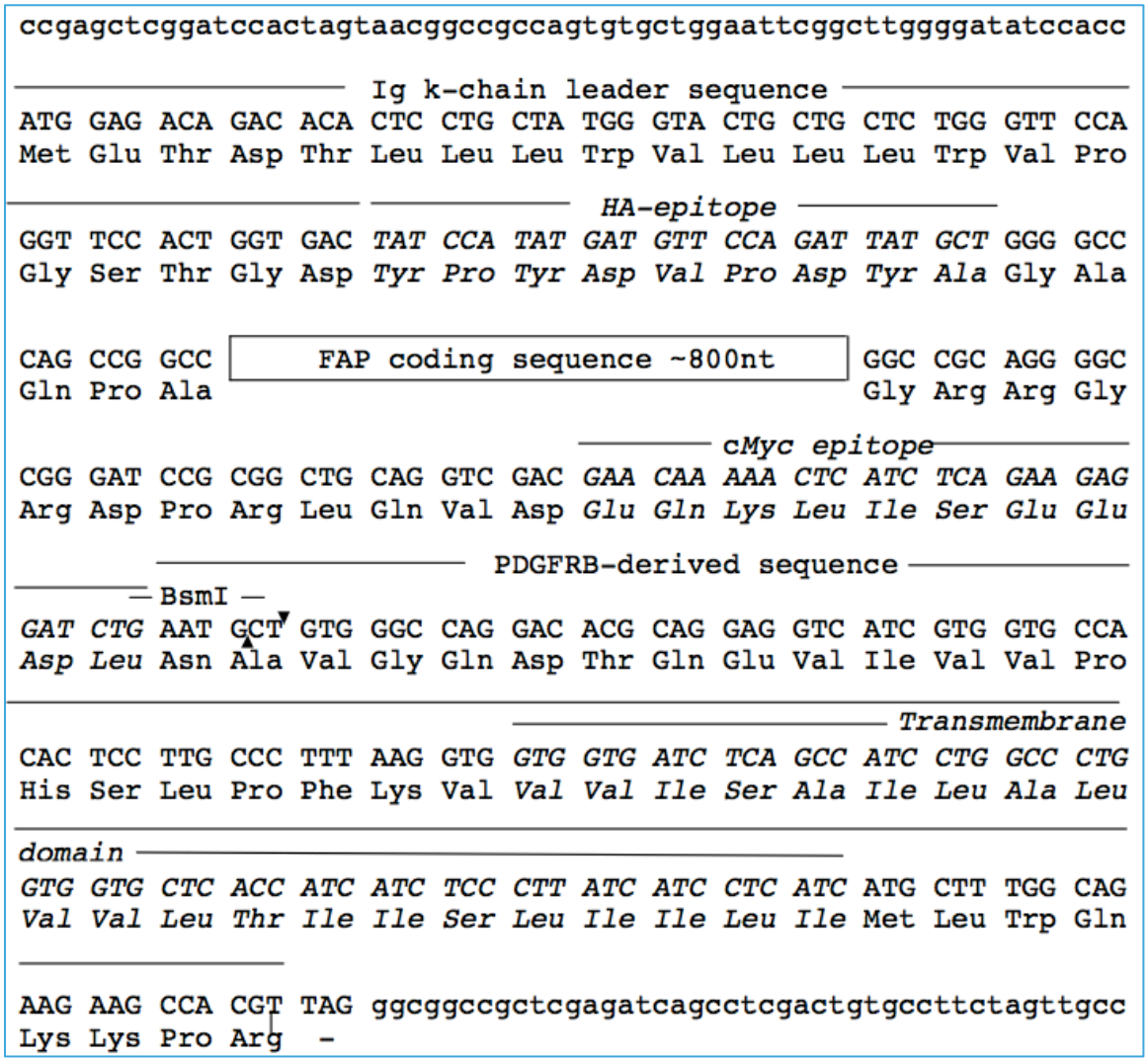
Soluble protein with stop codon

If the ORF encodes a soluble protein and ends with a stop codon, a fusion protein with an N-terminal FAP-tag is secreted into the medium.

Soluble protein with no stop codon

If the ORF encodes a soluble protein and does not end with a stop codon, a membrane-anchored fusion protein with an N-terminal FAP-tag is produced.

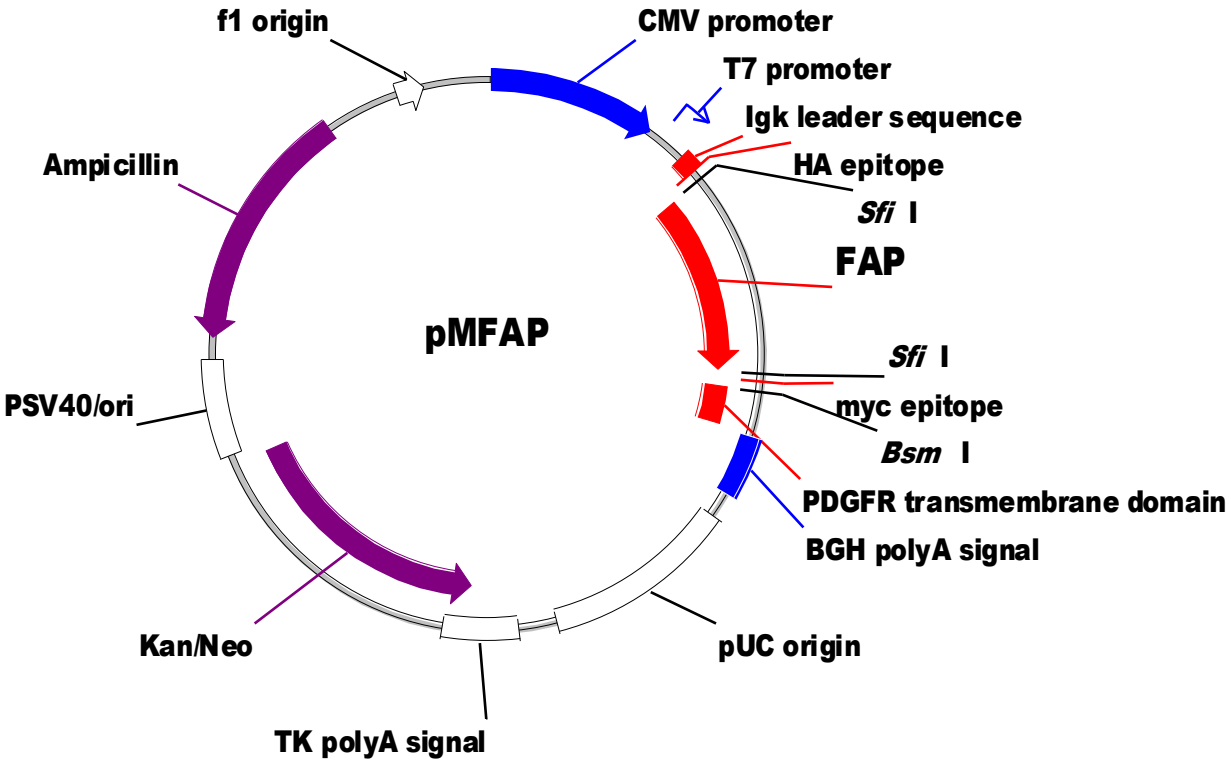
The translation unit in pMFAP1 vector series is represented below. Complete vector sequences can be accessed at www.spectragenetics.com



ORFs may be prepared for insertion into the BsmI site by PCR, using primers that add the sequence GAATG$_{\wedge}$CT^{V} (BsmI site GAATGC followed by T) at both termini. If the ORF contains an internal BsmI site, a number of other restriction enzymes can be used to provide the necessary 3' CT extension on the sense strand and the 3' AG extension on the antisense strand. These include BsrDI (GCAATG$_{\wedge}$NN^{V}), BtsI (GCAGTG$_{\wedge}$NN^{V}), BtsCI (GGATG$_{\wedge}$NN^{V}) and DrdI (GACNN$_{\wedge}$NN^{V}NNGTC).

pMFAP vector map

Complete sequences of pMFAP vectors can be accessed at www.spectragenetics.com



In addition to FAP-tagging vectors, SpectraGenetics also offers a wide-range of custom services as well as ready-to-use FAP-tagged GPCR's to expedite your research. Call us at 412-488-9350 or visit our website to learn what we can do for you.

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