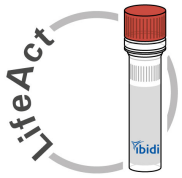


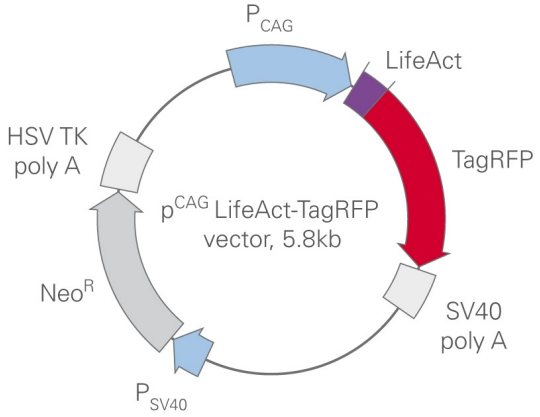
LifeAct is a 17 amino acids long fragment of a protein originating from *Saccharomyces cerevisiae*, which comprises an actin–binding domain. This marker can be used in various eukaryotic cells to stain filamentous actin (F–actin). Used in living cells it is perfectly labeling the highly dynamic F–actin and moreover, does not interfere with cellular processes.

Vector description

p^{CAG}LifeAct–TagRFP is a mammalian expression vector encoding LifeAct–TagRFP fusion protein. The vector can be used for fluorescent labeling of the actin cytoskeleton in various living cells. TagRFP codon usage is optimized for high expression in mammalian cells, i.e. humanized [Haas et al., 1996]. Actin–binding domain of the yeast protein Abp140 is fused to the TagRFP N-terminus [Riedl et al., 2008]. For more information on the reporter please visit www.evrogen.com. p^{CAG}LifeAct–TagRFP vector can be used as a source of LifeAct–TagRFP hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice (XhoI, NotI).

The vector backbone also contains the cytomegalovirus immediate early enhancer coupled to chicken β–actin promoter (CAG) [Niwa et al. 1991] for protein expression and SV40 polyadenylation signals (SV40 poly A) for proper processing of the 3′ end of the reporter mRNA. SV40 early promoter (P_{SV40}) provides neomycin resistance gene (Neo^R) expression to select stably transfected eukaryotic cells using G418. Neo^R gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Specifications



Packaging and storage	
Amount	20 µg dissolved in 40 µl TE
Concentration	500 ng/µl
Shipping conditions	+2 - 8°C
Storage conditions	-20°C *
Shelf life	Under proper storage conditions as indicated on vial.
TagRFP fluorescence	
Ex.$_{max}$	555 nm
Em.$_{max}$	584 nm
Find more information on www.evrogen.com.	

*Avoid repeated freeze and thaw cycles.

Location of features

P_{CAG}: 365-1704
CMV IE enhancer: 1-364
TATA box: 616-621

LifeAct: 1717-1767

TagRFP
Startcodon (ATG): 1789-1791
Stopcodon: 2500-2502

SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2655-2660 & 2684-2689
mRNA3'ends: 2690 & 2702

SV40 early promoter
Enhancer (72-bp tandem repeats): 3378-3449 & 3450-3521
21-bp repeats: 3525-3545, 3546-3566 & 3568-3588
Early promoter element: 3601-3607

Neomycin resistance gene (Neo^R)
Neomycin phosphotransferase coding sequences:
Startcodon (ATG): 3732-3734
Stopcodon: 4524-4526

Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4759-4764 & 4772-4777

Expression in mammalian cells

p^{CAG}LifeAct–TagRFP can be transfected into mammalian cells by any known transfection method. CAG promoter provides strong, constitutive expression of the LifeAct–TagRFP fusion in eukaryotic cells. If required, stable transformants can be selected using G418 [Gorman, 1985].

Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1–Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.

References

Gorman, High efficiency gene transfer into mammalian cells. In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), 1985: 143–90

Haas et al., Codon usage limitation in the expression of HIV–1 envelope glycoprotein. Curr Biol, 1996, 6 (3): 315–324

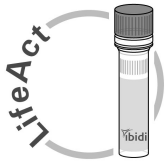
Niwa et al., Efficient selection for high-expression transfectants with a novel eukaryotic vector. Gene, 1991, 108: 193–200

Riedl et al., LifeAct: a versatile marker to visualize F–actin. Nature Methods, 2008, 5 (7): 605–607

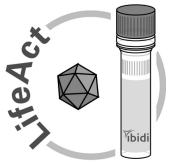
Note:

The vector sequence has been compiled using the information from sequence databases, and published literature, together with partial sequences obtained by ibidi. This vector has not been completely sequenced.

LifeAct family



Ordering number	Labeling	Amount	Vector type
60101	p^{CMV}LifeAct–TagGFP2	20 µg	plasmid
60102	p^{CMV}LifeAct–TagRFP	20 µg	plasmid
60106	p^{CAG}LifeAct–TagGFP2	20 µg	plasmid
60107	p^{CAG}LifeAct–TagRFP	20 µg	plasmid



Ordering number	Labeling	Amount	Vector type
60121	rAV^{CMV}–LifeAct–TagGFP2	1 × 10^9 IU	adenovirus
60122	rAV^{CMV}–LifeAct–TagRFP	1 × 10^9 IU	adenovirus
60124	rAV^{CAG}–LifeAct–TagRFP	1 × 10^9 IU	adenovirus

For research use only!

Further technical specifications can be found at www.ibidi.com. For questions and suggestions please contact us by e–mail *info@ibidi.de* or by telephone +49 (0)89/520 4617 0. All products are developed and produced in Germany.

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