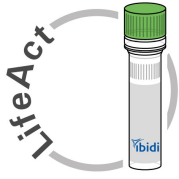


## Instructions

## p<sup>CAG</sup>LifeAct-TagGFP2



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.

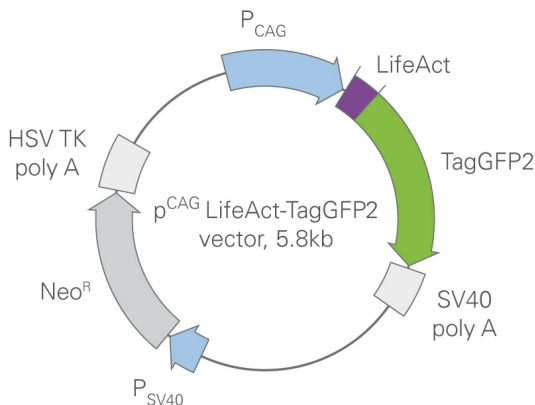
### Vector description

p<sup>CAG</sup>LifeAct-TagGFP2 is a mammalian expression vector encoding LifeAct-TagGFP2 fusion protein. The vector can be used for fluorescent labeling of the actin cytoskeleton in various living cells. TagGFP2 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 TagGFP2 N-terminus [Riedl et al., 2008]. For more information on the reporter please visit [www.evrogen.com](http://www.evrogen.com). p<sup>CAG</sup>LifeAct-TagGFP2 vector can be used as a source of LifeAct-TagGFP2 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  $\beta$ -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<sub>SV40</sub>) provides neomycin resistance gene (Neo<sup>R</sup>) expression to select stably transfected eukaryotic cells using G418. Neo<sup>R</sup> gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

### Specifications



### Location of features

P<sub>CAG</sub>: 365-1704  
 CMV IE Enhancer: 1-364  
 TATA box: 616-621

LifeAct: 1717-1767

TagGFP2  
 Startcodon (ATG): 1789-1791  
 Stopcodon: 2503-2505

SV40 early mRNA polyadenylation signal  
 Polyadenylation signals: 2658-2663 & 2687-2692  
 mRNA 3' ends: 2696 & 2708

SV40 early promoter  
 Enhancer (72-bp tandem repeats): 3384-3455 & 3456-3527  
 21-bp repeats: 3531-3551, 3552-3572 & 3574-3594  
 Early promoter element: 3606-3612

Neomycin resistance gene (Neo<sup>R</sup>)  
 Neomycin phosphotransferase coding sequences:  
 Startcodon (ATG): 3735-3737  
 Stopcodon: 4527-4529

Herpes simplex virus (HSV) thymidine kinase (TK)  
 polyadenylation signal  
 Polyadenylation signals: 4765-4770 & 4778-4783

#### Packaging and storage

Amount	20 $\mu$ g dissolved in 40 $\mu$ l TE
Concentration	500 ng/ $\mu$ l
Shipping conditions	+2 - 8°C
Storage conditions	-20°C *
Shelf life	Under proper storage conditions as indicated on vial.

#### TagGFP2 fluorescence

Ex <sub>max</sub>	483 nm
Em <sub>max</sub>	506 nm
Find more information on <a href="http://www.evrogen.com">www.evrogen.com</a> .	

\*Avoid repeated freeze and thaw cycles.

## Expression in mammalian cells

p<sup>CAG</sup>LifeAct–TagGFP2 can be transfected into mammalian cells by any known transfection method. CAG promoter provides strong, constitutive expression of the LifeAct–TagGFP2 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

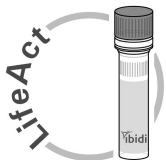
Niwa et al., Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene*, 1991, 108: 193–200

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

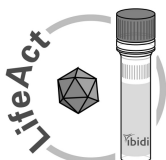
### Note:

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 <sup>CMV</sup> LifeAct–TagGFP2	20 µg	plasmid
60102	p <sup>CMV</sup> LifeAct–TagRFP	20 µg	plasmid
60106	p <sup>CAG</sup> LifeAct–TagGFP2	20 µg	plasmid
60107	p <sup>CAG</sup> LifeAct–TagRFP	20 µg	plasmid



Ordering number	Labeling	Amount	Vector type
60121	rAV <sup>CMV</sup> –LifeAct–TagGFP2	1 × 10 <sup>9</sup> IU	adenovirus
60122	rAV <sup>CMV</sup> –LifeAct–TagRFP	1 × 10 <sup>9</sup> IU	adenovirus
60124	rAV <sup>CAG</sup> –LifeAct–TagRFP	1 × 10 <sup>9</sup> IU	adenovirus

## For research use only!

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

© ibidi GmbH, Am Klopferspitz 19, 82152 Martinsried, Germany.

### Notice to Purchaser:

Evrogen Fluorescent Proteins TagGFP2 and TagRFP: This product is for internal non-commercial research use only. No rights are conveyed to modify or clone the gene encoding fluorescent protein contained in this product. The right to use this product specifically excludes the right to validate or screen compounds. For information on commercial licensing, contact Evrogen Licensing Department, email: [license@evrogen.com](mailto:license@evrogen.com).

LifeAct product and its variants: Use of this product is covered by the following patent application: WO2009068295 (A1). Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or ibidi Customer Service at +49 (0)89/520 4617 0. At its discretion, ibidi grants Not-For-Profit Entities a non-exclusive, personal, limited license to use this product for non-commercial life science research use only. Such license specifically excludes the right to sell or otherwise transfer this product, its components or derivatives thereof to third parties. No modifications to the protein coding sequence may be made without express written permission from ibidi. Any other use of this product requires a license from ibidi. For-Profit Entities wishing to use this product are required to obtain a license from ibidi. For license information, please contact a licensing representative by e-mail at [licensing@ibidi.com](mailto:licensing@ibidi.com).