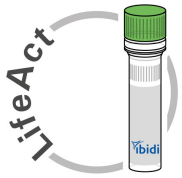


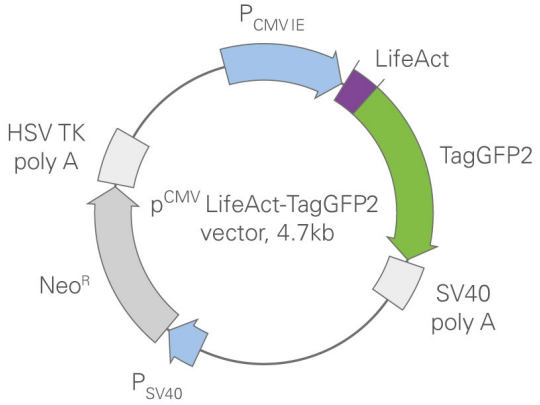
LifeAct® is a 17 amino acids long fragment of a protein originating from *Saccharomyces cerevisiae*, which comprises an actin–binding domain. This marker can be used in various eukaryotic cells to stain filamentous actin (F–actin). Used in living cells it is perfectly labeling the highly dynamic F–actin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

Vector Description

p^{CMV}–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. p^{CMV}–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 immediate early promoter of cytomegalovirus ($P_{CMV\,IE}$) 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



TagGFP2 Fluorescence	
Ex.$_{max}$	483 nm
Em.$_{max}$	506 nm
Find more information on www.evrogen.com.	

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.

*Avoid repeated freeze and thaw cycles.

Location of Features

$P_{CMV\,IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560

LifeAct®: 619-669

TagGFP2
Startcodon: 691-693
Stopcodon: 1406-1408

SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1560-1565 & 1589-1594
mRNA3′ends: 1598 & 1610

SV40 early promoter
Enhancer (72-bp tandem repeats): 2286-2357 & 2358-2429
21-bp repeats: 2433-2453, 2454-2474 & 2476-2496
Early promoter element: 2508-2514

Neomycin resistance gene (Neo®)
Neomycin phosphotransferase coding sequences:
Startcodon: 2637-2639
Stopcodon: 3429-3431

Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3667-3672 & 3680-3685

Expression in Mammalian Cells

p^{CMV}–LifeAct®–TagGFP2 can be transfected into mammalian cells by any known transfection method. CMV 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

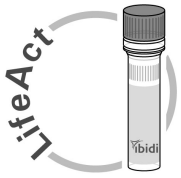
Riedl et al., LifeAct: a versatile marker to visualize F–actin. Nature Methods, 2008, 5 (7): 605–607

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.

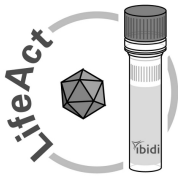
Ordering Information

LifeAct® Plasmids



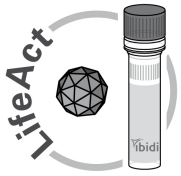
Cat. No.	Description	Amount
60101	**p^{CMV}–LifeAct®–TagGFP2**: plasmid, ready to use, 500 ng/µl	20 µg
60102	**p^{CMV}–LifeAct®–TagRFP**: plasmid, ready to use, 500 ng/µl	20 µg
60106	**p^{CAG}–LifeAct®–TagGFP2**: plasmid, ready to use, 500 ng/µl	20 µg
60107	**p^{CAG}–LifeAct®–TagRFP**: plasmid, ready to use, 500 ng/µl	20 µg

LifeAct® Adenoviral Vectors



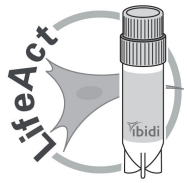
Cat. No.	Description	Amount
60121	**rAV^{CMV}–LifeAct®–TagGFP2**: adenoviral vector, ready to use, 1×10^{10} IU/ml	1×10^9 IU
60122	**rAV^{CMV}–LifeAct®–TagRFP**: adenoviral vector, ready to use, 1×10^{10} IU/ml	1×10^9 IU

LifeAct® Lentiviral Vectors



Cat. No.	Description	Amount
60141	**rLV^{Ubi}–LifeAct®–TagGFP2**: lentiviral vector, ready to use, 1×10^7 TU/ml	1×10^6 TU
60142	**rLV^{Ubi}–LifeAct®–TagRFP**: lentiviral vector, ready to use, 1×10^7 TU/ml	1×10^6 TU

LifeAct® Cell Lines



Cat. No.	Description	Amount
40101	**HT-1080 LifeAct®–TagGFP2**: HT-1080 cells expressing LifeAct®–TagGFP2	5×10^5 cells

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