

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.

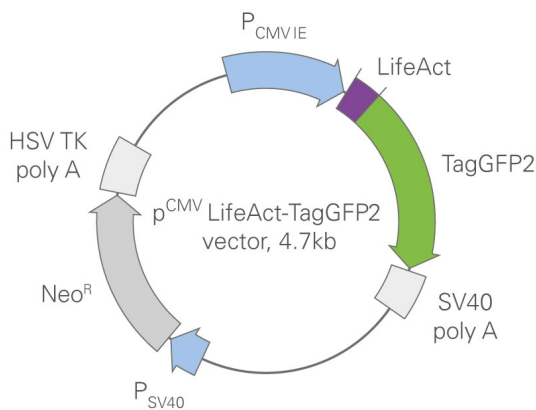
Vector Description

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. Actin-binding domain of the yeast protein Abp140 is fused to the TagGFP2 N-terminus. 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 back-

bone 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_{CMVIE}) 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



Location of Features

P_{CMVIE}: 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

TagGFP2 Fluorescence

Ex. _{max}	483 nm
Em. _{max}	506 nm

Find more information on www.evrogen.com.

Packaging and Storage

Amount	20 µg, lyophilized
Shipping conditions	room temperature
Storage conditions	-20°C*
Shelf life	Under proper storage conditions as indicated on vial.

*Avoid repeated freeze and thaw cycles.

Plasmid Reconstitution

Spin the tube containing the lyophilized plasmid. To obtain a plasmid solution with a concentration of 1 µg/µl, resuspend the DNA in 20 µl of TE-buffer or sterile water. Store resuspended plasmid at -20°C.

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.

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.

Note:

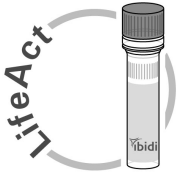
The vector sequence has been compiled using the information from sequence databases, and published literature, together with partial sequences obtained by ibidi. This vector has not been completely sequenced.

Selected References

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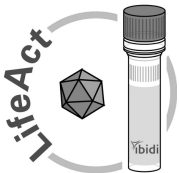
Ordering Information

LifeAct[®] Plasmids



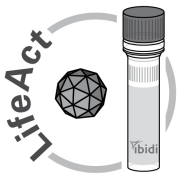
Cat. No.	Description	Amount
60101	p ^{CMV} -LifeAct [®] -TagGFP2: plasmid, lyophilized	20 µg
60102	p ^{CMV} -LifeAct [®] -TagRFP: plasmid, lyophilized	20 µg
60106	p ^{CAG} -LifeAct [®] -TagGFP2: plasmid, lyophilized	20 µg
60107	p ^{CAG} -LifeAct [®] -TagRFP: plasmid, lyophilized	20 µg

LifeAct[®] Adenoviral Vectors



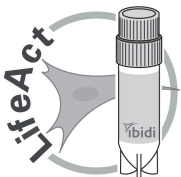
Cat. No.	Description	Amount
60121	rAV ^{CMV} -LifeAct [®] -TagGFP2: adenoviral vector, ready to use, 1 × 10 ¹⁰ IU/ml	1 × 10 ⁹ IU
60122	rAV ^{CMV} -LifeAct [®] -TagRFP: adenoviral vector, ready to use, 1 × 10 ¹⁰ IU/ml	1 × 10 ⁹ IU

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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