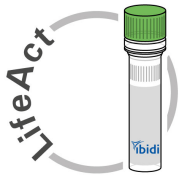


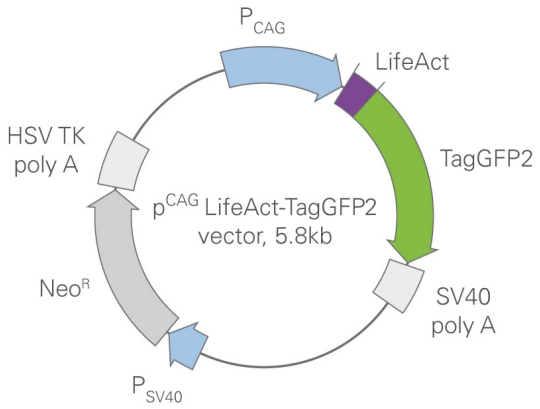

LifeAct® is a 17 amino acids long fragment of a protein originating from *Saccharomyces cerevisiae*, which comprises an actin–binding domain. This marker can be used in various eukaryotic cells to stain filamentous actin (F–actin). Used in living cells it is perfectly labeling the highly dynamic F–actin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

## Vector Description

$p^{CAG}$–LifeAct®–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. $p^{CAG}$–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 ($P_{SV40}$) 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

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

LifeAct®: 1717-1767

TagGFP2
Startcodon: 1789-1791
Stopcodon: 2503-2505

SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2658-2663 & 2687-2692
mRNA3′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®)
Neomycin phosphotransferase coding sequences:
Startcodon: 3735-3737
Stopcodon: 4527-4529

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

## Expression in Mammalian Cells

p$^{CAG}$–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

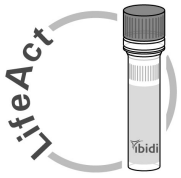
Riedl et al., LifeAct: a versatile marker to visualize F–actin. Nature Methods, 2008, 5 (7): 605–607

### 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.

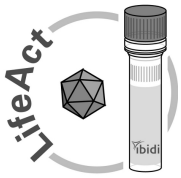
## Ordering Information

### LifeAct® Plasmids



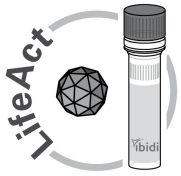

| Cat. No. | Description | Amount |
|---|---|---|
| 60101 | **$p^{CMV}$–LifeAct®–TagGFP2**: plasmid, ready to use, 500 ng/µl | 20 µg |
| 60102 | **$p^{CMV}$–LifeAct®–TagRFP**: plasmid, ready to use, 500 ng/µl | 20 µg |
| 60106 | **$p^{CAG}$–LifeAct®–TagGFP2**: plasmid, ready to use, 500 ng/µl | 20 µg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

### LifeAct® Adenoviral Vectors



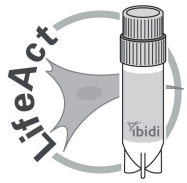

| Cat. No. | Description | Amount |
|---|---|---|
| 60121 | **$rAV^{CMV}$–LifeAct®–TagGFP2**: adenoviral vector, ready to use, $1 \times 10^{10}$ IU/ml | $1 \times 10^9$ IU |
| 60122 | **$rAV^{CMV}$–LifeAct®–TagRFP**: adenoviral vector, ready to use, $1 \times 10^{10}$ IU/ml | $1 \times 10^9$ IU |

### LifeAct® Lentiviral Vectors




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

### LifeAct® Cell Lines




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

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