

LifeAct<sup>®</sup> 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.

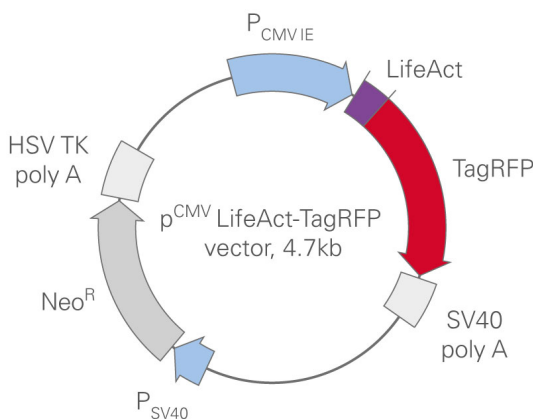
## Vector Description

p<sup>CMV</sup>-LifeAct<sup>®</sup>-TagRFP is a mammalian expression vector encoding LifeAct<sup>®</sup>-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](http://www.evrogen.com). p<sup>CMV</sup>-LifeAct<sup>®</sup>-TagRFP vector can be used as a source of LifeAct<sup>®</sup>-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 immediate early promoter of cytomegalovirus (P<sub>CMVIE</sub>) 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>CMVIE</sub>: 1-589  
 Enhancer region: 59-465  
 TATA box: 554-560

LifeAct<sup>®</sup>: 619-669

TagRFP  
 Startcodon: 691-693  
 Stopcodon: 1403-1405

SV40 early mRNA polyadenylation signal  
 Polyadenylation signals: 1554-1559 & 1583-1588  
 mRNA3'ends: 1592 & 1604

SV40 early promoter  
 Enhancer (72-bp tandem repeats): 2280-2351 & 2352-2423  
 21-bp repeats: 2427-2447, 2448-2468 & 2470-2490  
 Early promoter element: 2503-2509

Neomycin resistance gene (Neo<sup>R</sup>)  
 Neomycin phosphotransferase coding sequences:  
 Startcodon: 2634-2636  
 Stopcodon: 3426-3428

Herpes simplex virus (HSV) thymidine kinase (TK)  
 polyadenylation signal  
 Polyadenylation signals: 3661-3666 & 3674-3679

### TagRFP Fluorescence

Ex. <sub>max</sub>	555 nm
Em. <sub>max</sub>	584 nm
Find more information on <a href="http://www.evrogen.com">www.evrogen.com</a> .	

### 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.

## Expression in Mammalian Cells

p<sup>CMV</sup>-LifeAct<sup>®</sup>-TagRFP can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the LifeAct<sup>®</sup>-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

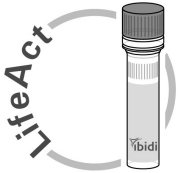
- Gorman, High efficiency gene transfer into mammalian cells. In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), 1985: 143–90
- Haas et al., Codon usage limitation in the expression of HIV-1 envelope glycoprotein. *Curr Biol*, 1996, 6 (3): 315–324
- Riedl et al., 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.

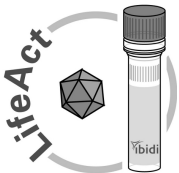
## Ordering Information

### LifeAct<sup>®</sup> Plasmids



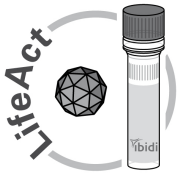
Cat. No.	Description	Amount
60101	p <sup>CMV</sup> -LifeAct <sup>®</sup> -TagGFP2: plasmid, ready to use, 500 ng/μl	20 μg
60102	p <sup>CMV</sup> -LifeAct <sup>®</sup> -TagRFP: plasmid, ready to use, 500 ng/μl	20 μg
60106	p <sup>CAG</sup> -LifeAct <sup>®</sup> -TagGFP2: plasmid, ready to use, 500 ng/μl	20 μg
60107	p <sup>CAG</sup> -LifeAct <sup>®</sup> -TagRFP: plasmid, ready to use, 500 ng/μl	20 μg

### LifeAct<sup>®</sup> Adenoviral Vectors



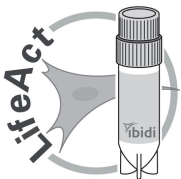
Cat. No.	Description	Amount
60121	rAV <sup>CMV</sup> -LifeAct <sup>®</sup> -TagGFP2: adenoviral vector, ready to use, 1 × 10 <sup>10</sup> IU/ml	1 × 10 <sup>9</sup> IU
60122	rAV <sup>CMV</sup> -LifeAct <sup>®</sup> -TagRFP: adenoviral vector, ready to use, 1 × 10 <sup>10</sup> IU/ml	1 × 10 <sup>9</sup> IU

### LifeAct<sup>®</sup> Lentiviral Vectors



Cat. No.	Description	Amount
60141	rLV <sup>Ubi</sup> -LifeAct <sup>®</sup> -TagGFP2: lentiviral vector, ready to use, 1 × 10 <sup>7</sup> TU/ml	1 × 10 <sup>6</sup> TU
60142	rLV <sup>Ubi</sup> -LifeAct <sup>®</sup> -TagRFP: lentiviral vector, ready to use, 1 × 10 <sup>7</sup> TU/ml	1 × 10 <sup>6</sup> TU

### LifeAct<sup>®</sup> Cell Lines



Cat. No.	Description	Amount
40101	HT-1080 LifeAct <sup>®</sup> -TagGFP2: HT-1080 cells expressing LifeAct <sup>®</sup> -TagGFP2	5 × 10 <sup>5</sup> cells

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