

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.

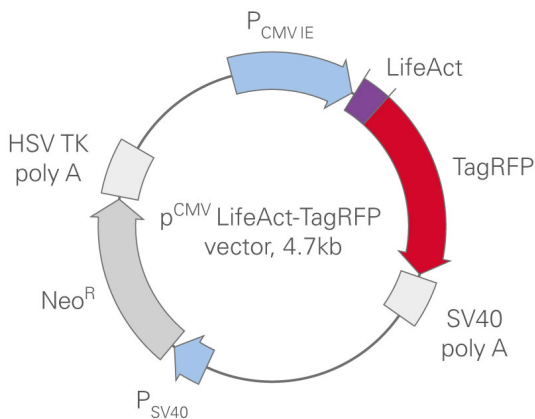
Vector Description

p^{CMV}-LifeAct®-TagRFP is a mammalian expression vector encoding LifeAct®-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. Actin-binding domain of the yeast protein Abp140 is fused to the TagRFP N-terminus. For more information on the reporter please visit www.evrogen.com. p^{CMV}-LifeAct®-TagRFP vector can be used as a source of LifeAct®-TagRFP 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

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®)
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. _{max}	555 nm
Em. _{max}	584 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[®]-TagRFP can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the LifeAct[®]-TagRFP 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.

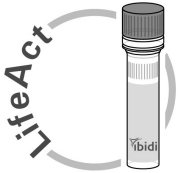
Note:

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.

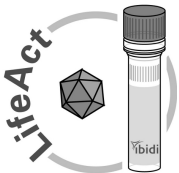
Selected References

- J. Bae, B. H. Sung, I. H. Cho, and W. K. Song. F-Actin-Dependent Regulation of NESH Dynamics in Rat Hippocampal Neurons. *PLoS ONE*, 2012. doi: 10.1371/journal.pone.0034514.
- E. Chin, K. Kirker, M. Zuck, G. James, and K. Hybiske. Actin Recruitment to the Chlamydia Inclusion Is Spatiotemporally Regulated by a Mechanism That Requires Host and Bacterial Factors. *PLoS ONE*, 2012. doi: 10.1371/journal.pone.0046949.
- A. D. Doyle, N. Carvajal, A. Jin, K. Matsumoto, and K. M. Yamada. Local 3D matrix microenvironment regulates cell migration through spatiotemporal dynamics of contractility-dependent adhesions. *Nature Communications*, 2015. doi: 10.1038/ncomms9720.
- S. Inoue, V. Frank, M. Hoerning, S. Kaufmann, H. Y. Yoshikawa, J. P. Madsen, A. L. Lewis, S. P. Armes, and M. Tanaka. Live cell tracking of symmetry break in actin cytoskeleton triggered by abrupt changes in micromechanical environments. *Biomaterials Science*, 2015. doi: 10.1039/c5bm00205b.
- J. Riedl, A. H. Crevenna, K. Kessenbrock, J. H. Yu, D. Neukirchen, M. Bista, F. Bradke, D. Jenne, T. A. Holak, Z. Werb, M. Sixt, and R. Wedlich-Soldner. Lifeact: a versatile marker to visualize f-actin. *Nature Methods*, 5(7):605–607, 2008. doi: 10.1038/nmeth.1220.
- M. Stölting, C. Wiesner, V. van Vliet, E. Butt, H. Pavenstädt, S. Linder, and J. Kremerskothen. Lasp-1 Regulates Podosome Function. *PLoS ONE*, 2012. doi: 10.1371/journal.pone.0035340.
- J. Villanueva, V. Torres, C. J. Torregrosa-Hetland, V. Garcia-Martinez, I. Lopez-Font, S. Viniegra, and L. M. Gutierrez. F-Actin-Myosin II Inhibitors Affect Chromaffin Granule Plasma Membrane Distance and Fusion Kinetics by Retraction of the Cytoskeletal Cortex. *Journal of Molecular Neuroscience*, 2012. doi: 10.1007/s12031-012-9800-y.
- J. Wu, I. A. Kent, N. Shekhar, T. J. Chancellor, A. Mendonca, R. B. Dickinson, and T. P. Lele. Actomyosin Pulls to Advance the Nucleus in a Migrating Tissue Cell. *Biophysical Journal*, 2014. doi: 10.1016/j.bpj.2013.11.4489.
- R. Wuestefeld, J. Chen, K. Meller, B. Brand-Saberi, and C. Theiss. Impact of vegf on astrocytes: Analysis of gap junctional intercellular communication, proliferation, and motility. *Glia*, 2012. doi: 10.1002/glia.22325.
- A. Yoshida, N. Sakai, Y. Uekusa, Deguchi K., J. L. Gilmore, M. Kumeta, S. Ito, and K. Takeyasu. Probing in vivo dynamics of mitochondria and cortical actin networks using high-speed atomic force/fluorescence microscopy. *Genes To Cells*, 2015. doi: 10.1111/gtc.12204.

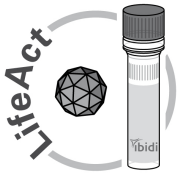
Ordering Information

 LifeAct[®] Plasmids


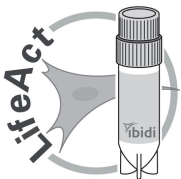
Cat. No.	Description	Amount
60101	p ^{CMV} -LifeAct [®] -TagGFP2: plasmid, lyophilized	20 µg
60102	p ^{CMV} -LifeAct [®] -TagRFP: plasmid, lyophilized	20 µg
60106	p ^{CAG} -LifeAct [®] -TagGFP2: plasmid, lyophilized	20 µg
60107	p ^{CAG} -LifeAct [®] -TagRFP: plasmid, lyophilized	20 µg

 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

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.

LifeAct® Product and its Variants

Use of these products and product applications are covered by european and foreign patents and patents pending.

All names containing the designation ® are registered with the Office for Harmonization in the Internal Market and the U.S. Patent and Trademark Office.

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 email at licensing@ibidi.com.

For research use only!

Further technical specifications can be found at www.ibidi.com. For questions and suggestions please contact us by e-mail 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.