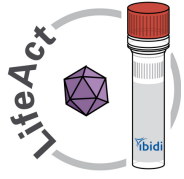


## Instructions

## rAV<sup>CAG</sup>-LifeAct-TagRFP



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.

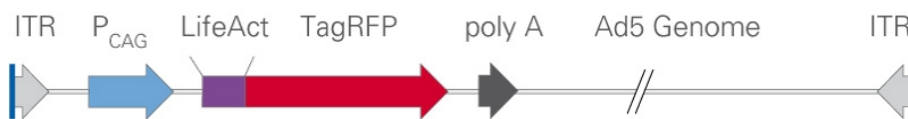
### Vector description

Using adenoviral vectors to introduce genetic material into host cells has numerous advantages. Recombinant adenoviruses are especially applicable for gene transfer and protein expression in cells that have low transfection efficiency with standard transfection methods.

Various mammalian cell types (both replicative and non-replicative) can be infected with adenoviral vectors for high expression of a recombinant protein. After transduction, the virus stays epichromosomal, meaning not integrating into the host genome [Barnett 2002, Medina-Kauwe 2003].

rAV<sup>CAG</sup>-LifeAct-TagRFP is a E1/E3-deleted, human Adenovirus (Serotyp 5) comprising LifeAct-TagRFP fusion protein. The vector can be used for fluorescent labeling of the actin cytoskeleton in various living cells with special focus on primary 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). For strong expression of the fusion protein in mammalian cells the vector contains the cytomegalovirus immediate early enhancer coupled to chicken  $\beta$ -actin promoter (CAG) [Niwa et al. 1991]. SV40 polyadenylation signals (SV40 poly A) direct proper processing of the 3' end of the reporter mRNA.

### Vector Map



ITR: Inverted terminal repeat  
P<sub>CAG</sub>: CMV IE/Chicken  $\beta$ -actin promoter  
Transgene: LifeAct-TagRFP  
polyA: SV40 polyadenylation signal  
Ad5 Genome: Adenovirus serotype 5 genome (E1/E3-deleted)

### Specifications

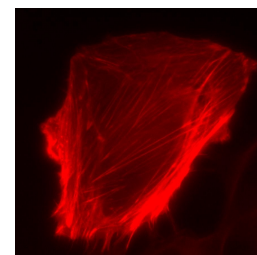
Packaging and storage	
Amount	100 $\mu$ l (25 mM Tris, pH 7.5, 2.5 mM MgCl <sub>2</sub> , 1 M NaCl, 10% glycerol)
Concentration	1 $\times$ 10 <sup>10</sup> IU/ml
Shipping conditions	dry ice
Storage conditions	-80°C *
Shelf life	Under proper storage conditions as indicated on vial.
TagRFP fluorescence	
Ex. <sub>max</sub>	555 nm
Em. <sub>max</sub>	584 nm

Find more information on [www.evrogen.com](http://www.evrogen.com).

\*Avoid repeated freeze and thaw cycles.  
If needed aliquot and store at -80°C

### Expression in mammalian cells

As the amount of adenovirus cell surface receptors diverges wildly among different cell types the optimal concentration of adenoviral vectors to use varies greatly. A range of 10-200 MOI (multiplicity of infection) is used for most cell types, but up to 1000 MOI may be used for lymphoid cell types.



A549 cells transduced with rAV-LifeAct-TagRFP (MOI 20) and imaged 48 hours later.

**References**

Barnett et al., Targeted adenoviral vectors. *BBA*, 2002, 1575: 1–14

Haas et al., Codon usage limitation in the expression of HIV-1 envelope glycoprotein. *Curr Biol*, 1996, 6 (3): 315–324

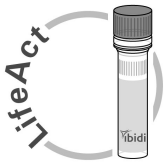
Medina-Kauwe, Endocytosis of adenovirus and adenovirus capsid proteins. *ADDR*, 2003, 55: 1485–1496

Niwa et al., Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene*, 1991, 108: 193–200

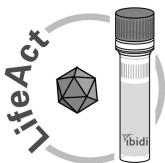
Riedl et al., LifeAct: a versatile marker to visualize F-actin. *Nature Methods*, 2008, 5 (7): 605–607

**Safety consideration:**

Note, that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. Always wear gloves, use filtered tips and work under a biosafety hood.

**LifeAct family**


Ordering number	Labeling	Amount	Vector type
60101	p <sup>CMV</sup> LifeAct-TagGFP2	20 µg	plasmid
60102	p <sup>CMV</sup> LifeAct-TagRFP	20 µg	plasmid
60106	p <sup>CAG</sup> LifeAct-TagGFP2	20 µg	plasmid
60107	p <sup>CAG</sup> LifeAct-TagRFP	20 µg	plasmid



Ordering number	Labeling	Amount	Vector type
60121	rAV <sup>CMV</sup> -LifeAct-TagGFP2	1 × 10 <sup>9</sup> IU	adenovirus
60122	rAV <sup>CMV</sup> -LifeAct-TagRFP	1 × 10 <sup>9</sup> IU	adenovirus
60124	rAV <sup>CAG</sup> -LifeAct-TagRFP	1 × 10 <sup>9</sup> IU	adenovirus

**For research use only!**

Further technical specifications can be found at [www.ibidi.com](http://www.ibidi.com). For questions and suggestions please contact us by e-mail [info@ibidi.de](mailto:info@ibidi.de) or by telephone +49 (0)89/520 4617 0. All products are developed and produced in Germany.

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