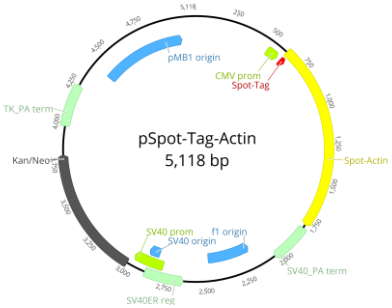
pSpot-Tag®-Actin plasmid for expression of Spot-Tag® *β*-actin fusion protein in mammalian cells



For plasmid sequence, please visit www.chromotek.com.

Product	Code	Size
pSpot-Tag-Actin	ev-31	10 µg

Vector type	Mammalian expression vector
Tag	Spot-Tag® (PDRVRAVSHWSS) N-terminal
Gene	Human *β*-actin
Promoter	CMV
Expression	Constitutive
Host cells	Mammalian cell lines (e.g. HeLa)
Selection	Kanamycin or neomycin
Replication	pMB1
Use	Expression of human *β*-actin fused to Spot-Tag® (N-terminal) in mammalian cells as a positive control.

Vector description

The plasmid pSpot-Tag®-Actin is a mammalian expression vector encoding human *β*-actin fused to the Spot-Tag®. The Spot-Tag® comprises the sequence PDRVRAVSHWSS and is fused to the N-terminus of *β*-actin.

This plasmid may be used as a positive control for immunofluorescence microscopy or Western blot experiments using the Spot-Tag® system.

Location of features

CMV promoter: 508-589

Spot-Tag®-actin: 613-1776

Spot-Tag®: 616-651

SV40 terminator: 1799-2043

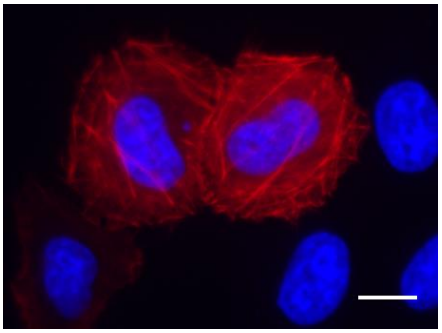
Kanamycin resistance gene: 3005-3805

pMB1 replication origin: 4400-5019

Expression in mammalian cells & detection

Transient, constitutive expression of Spot-Tag®-Actin is achieved by transfection of mammalian cells using any known transfection method, e.g. Lipofectamine 2000.

The resulting Spot-Tag® fusion protein may be imaged in immunofluorescence microscopy or detected in Western blotting using “Spot-Label ATTO488” or “Spot-Label ATTO594” (product code eba488 and eba594, respectively).



HeLa cells were transfected with the Spot-Tag®-Actin plasmid overnight, fixed, stained with Spot-Label ATTO594 (product code eba594), and imaged using 40X objective. Spot-Tag®-Actin filaments in red, cell nuclei in blue (DAPI). Scale bar 10 µm.

Propagation in *E. coli*

The DNA provided is suitable to be used directly in cell transfection. The plasmid may be propagated in *E. coli* using strains such as DH5alpha, HB101, XL1-Blue, and other general purpose strains. The vector confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.

Note: The plasmid DNA was isolated from *dam*$^+$ *E. coli*. In consequence, some restriction sites are blocked by methylation. If you wish to digest the vector using such sites, you will need to transform a *dam*$^-$ host using this plasmid and prepare fresh DNA.

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