



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

Location of features

T7 promoter: 1-19
 Lac operon: 19-46
 RBS: 64-80
 EGFP-Spot-Tag®: 88-843
 Spot-Tag®: 805-840
 rrnB terminator: 1025-1182
 Kanamycin resistance gene: 1462-2277
 pMB1 replication origin: 2445-3064

Product	Code	Size
pSpot2_GFP-Spot-Tag	ev-32	10 µg

Vector type	Bacterial expression vector
Tag	Spot-Tag® (PDRVRAVSHWSS) C-terminal
Gene	Enhanced green fluorescent protein (EGFP)
Promoter	T7
Induction	IPTG, lactose
Host cells	<i>Escherichia coli</i> DE3 strains
Selection	Kanamycin
Replication	pMB1
Use	Expression of EGFP fused to Spot-Tag® (C-terminal) in <i>E. coli</i> as a positive control.

Vector description

The plasmid pSpot2_GFP is a bacterial expression vector encoding *Aequorea victoria* Enhanced Green fluorescent protein (EGFP) fused to the Spot-Tag®. The Spot-Tag® comprises the sequence PDRVRAVSHWSS and is fused to the C-terminus of EGFP.

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

Expression in *E. coli* & detection

Protein expression can be induced using lactose or IPTG, but requires the use of DE3 prophage-positive *E. coli* expression strains.

The resulting Spot-Tag® fusion protein can be purified using the ChromoTek Spot-Trap® (product code eta or etma) or detected using Spot-Label ATTO488 or ATTO549 (product codes eba488 and eba594) in Western blotting.

Propagation in *E. coli*

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 (50 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.

Notice to Purchaser:

This plasmid was designed and generated by Dr. Philipp Kaiser from the Naturwissenschaftliches und Medizinisches Institut (NMI) at the University of Tübingen, Germany, and is distributed by ChromoTek GmbH. Please acknowledge Dr. Philipp Kaiser (NMI, Tübingen, Germany) and ChromoTek GmbH (Marinsried, Germany) when using or redistributing this vector.

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