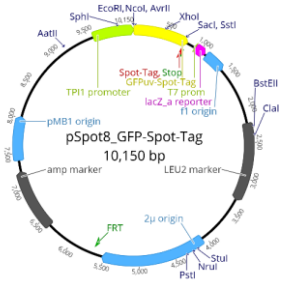
pSpot8_GFP-Spot-Tag plasmid for expression of GFP-Spot-Tag® fusion protein in *S. cerevisiae*



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

Location of features

TPI1 promoter: 9576-9

GFPuv-Spot-Tag®: 10-780

Spot-Tag®: 742-777

T7 primer site: 892-910

Leucin marker (LEU2): 2226-3332

2µ replication origin: 4058-5527

Ampicillin resistance gene: 6385-7245

pMB1 replication origin: 7400-8019

Product	Code	Size
pSpot8_GFP-Spot-Tag	ev-33	10 µg

Vector type	Yeast expression vector
Tag	Spot-Tag® (PDRVRAVSHWSS) C-terminal
Gene	Green fluorescent protein (GFP) variant GFPuv
Promoter	TPI1
Induction	Constitutive
Host cells	*Saccharomyces cerevisiae* (*leuΔ*)
Selection	Leucin (*S. cerevisiae*) Ampicillin (*E. coli*)
Replication	2µ (*S. cerevisiae*) pBR322 (*E. coli*)
Use	Expression of GFP fused to Spot-Tag® (C-terminal) in *S. cerevisiae* as a positive control

Vector description

The plasmid pSpot8_GFP-Spot-Tag is a *S. cerevisiae* expression vector encoding the *Aequorea victoria* Green fluorescent protein (GFP) variant GFPuv fused to the Spot-Tag®. The Spot-Tag® comprises the sequence PDRVRAVSHWSS and is fused to the C-terminus of GFPuv. This plasmid may be used as a positive control for immunoprecipitation or Western blot experiments using the Spot-Tag® system.

Expression in *S. cerevisiae* & detection

Transform a suitable *leu2* deletion strain of *S. cerevisiae* using the plasmid pSpot8_GFP-Spot-Tag and standard methods and grow on media lacking leucine. Protein expression is constitutive under the strong triosephosphate isomerase (TPI1) promoter.

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 ATTO594 (product code 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 ampicillin (100 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 (Martinsried, Germany) when using or redistributing this vector.
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