

For plasmid sequence, please visit [www.chromotek.com](http://www.chromotek.com).

### Location of features

TPI1 promoter: 7239-9  
 Start codon (ATG): 10-12  
 Spot-Tag®: 13-48  
 MCS: 47-112  
 T7 primer site: 230-248  
 Leucin marker (LEU2): 1564-2670  
 CEN replication origin: 3398-3916  
 Ampicillin resistance gene: 4048-4908  
 pBR322 replication origin: 5063-5682

Product	Code	Size
pSpot5	ev-5	1.25 µg
Vector type	yeast expression vector	
Tag	Spot-Tag® (PDRVRAVSHWSS) N-terminal	
MCS	PstI, BamHI, AvrII, Apal, HindIII, EcoRV, XhoI, XmaI, NheI, SacI	
Promoter	TPI1	
Induction	constitutive	
Host cells	<i>Saccharomyces cerevisiae</i> ( <i>leu</i> )	
Selection	leucin ( <i>S. cerevisiae</i> ) Ampicillin ( <i>E. coli</i> )	
Replication	CEN ( <i>S. cerevisiae</i> ) pBR322 ( <i>E. coli</i> )	
Use	Expression of a protein of interest fused to Spot-Tag® (N-terminal) in <i>S. cerevisiae</i> .	

### Vector description

The plasmid pSpot5 is an expression vector for Spot-Tag® fusion proteins in *S. cerevisiae*. After cloning the protein of interest (POI) into the multiple cloning site (MCS) provided by pSpot5, the Spot-Tag® (sequence: PDRVRAVSHWSS) will be fused to the N-terminus of the POI.

### Expression in *S. cerevisiae*

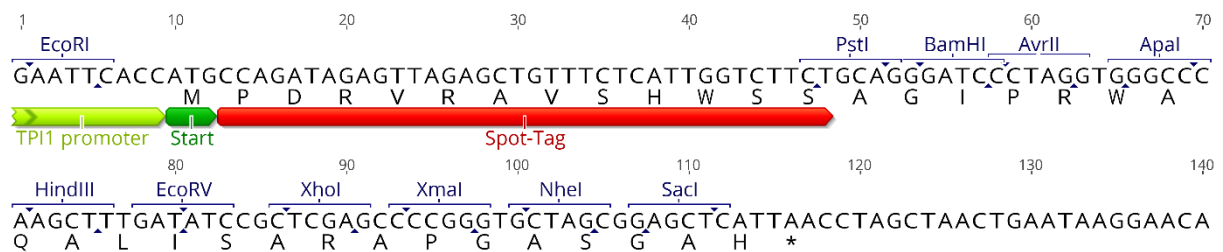
Transform a suitable *leu2* deletion strain of *S. cerevisiae* using pSpot5 and standard methods and grow on media lacking leucine. Protein expression is constitutive under the strong triosephosphate isomerase (TPI1) promoter. The resulting Spot-Tag® fusion protein can be purified using the ChromoTek Spot-Trap®.

### Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include 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*<sup>+</sup>-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites, you will need to transform the vector into a *dam*<sup>-</sup> host and make fresh DNA

### Multiple cloning site (MCS)



### 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. The development of this plasmid was supported by a grant "Zentrales Innovationsprogramm Mittelstand" (ZIM) from the Federal Ministry for Economic Affairs and Energy of Germany.