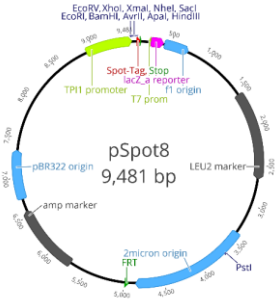
# pSpot8 plasmid for expression of C-terminal Spot-Tag® fusion proteins in *S. cerevisiae*

| Product | Code | Size |
|---|---|---|
| pSpot8 | ev-8 | 1.25 µg |




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

| | |
|---|---|
| Vector type | yeast expression vector |
| Tag | Spot-Tag® (PDRVRAVSHWSS)<br>C-terminal |
| MCS | BamHI, AvrII, ApaI, HindIII, EcoRV, XhoI, XmaI, NheI, SacI |
| Promoter | TPI1 |
| Induction | constitutive |
| Host cells | *Saccharomyces cerevisiae* (*leuΔ*) |
| Selection | leucin (*S. cerevisiae*)<br>ampicillin (*E. coli*) |
| Replication | 2µ (*S. cerevisiae*)<br>pBR322 (*E. coli*) |
| Use | Expression of a protein of interest fused to Spot-Tag® (C-terminal) in *S. cerevisiae*. |

## Location of features

TPI1 promoter: 8907-9

MCS: 14-73

Spot-Tag®: 73-108

Stop codon (TAA): 109-11

T7 primer site: 223-241

Leucin marker (LEU2): 1557-2663

2µ replication origin: 3389-4858

Ampicillin resistance gene: 5716-6576

pRB322 replication origin: 6731-7350

## Vector description

The plasmid pSpot8 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 pSpot8, the Spot-Tag® (sequence: PDRVRAVSHWSS) will be fused to the C-terminus of the POI.

## Expression in *S. cerevisiae*

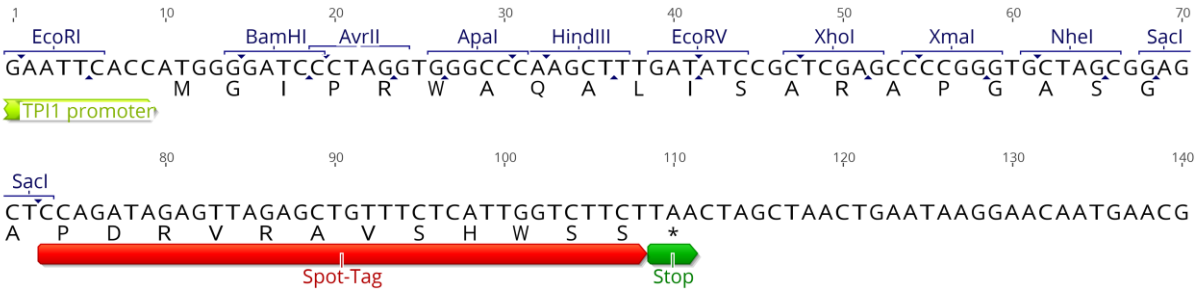
Transform a suitable *leu2* deletion strain of *S. cerevisiae* using pSpot8 and standard methods and grow on media lacking leucine. Protein expression is constitutive under the strong triosephosphate isomerase (TPI1) promoter. The resulting Spot-Tag® fusion protein can be purified using the ChromoTek 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$^+$-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$^-$ 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.
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