Expression Vector pEG-His1

Product Information Sheet
PEG01

SUMMARY

shipped at RT; store at 4 °C

For research use only

Product

pEG-His1 vector for IPTG inducible expression of gene products (potentially toxic) with C-terminal RGS motif and 6xHis-tag. 5' and 3' primers for sequencing are provided in addition.

Description

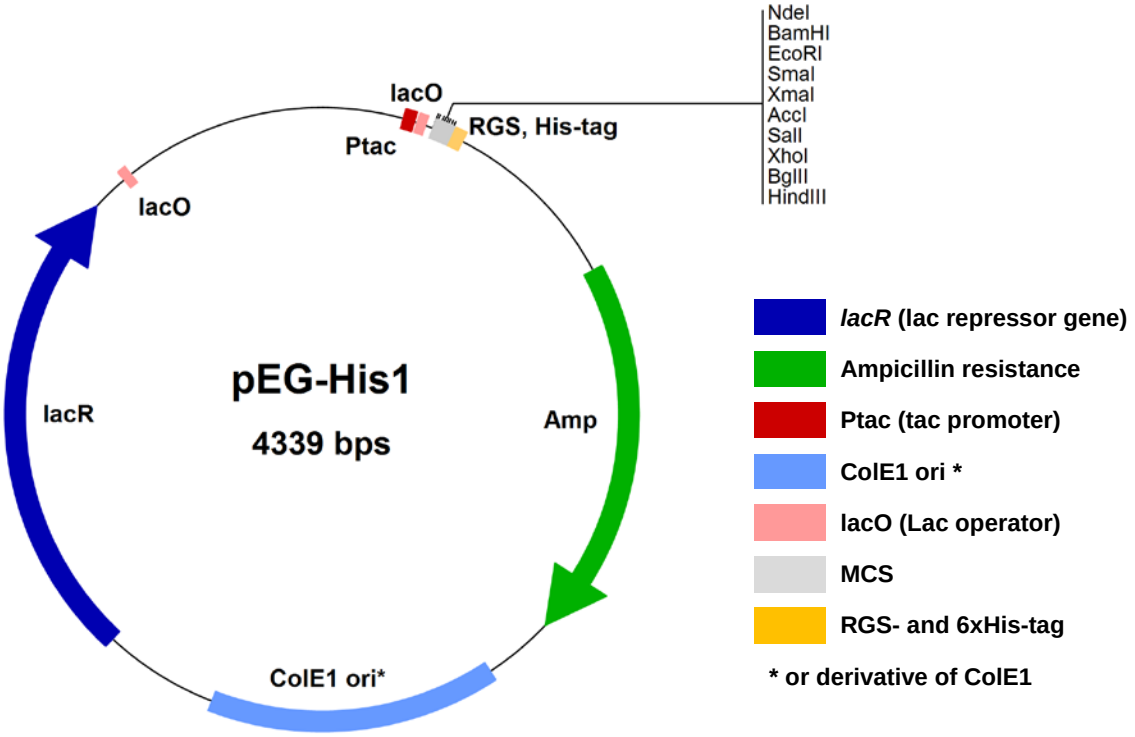
The pEG-His1 vector was constructed for the expression of potentially toxic gene products in *E. coli*. The tac promoter allows highly efficient gene expression after induction with IPTG. To obtain the exceptional tightness of the tac promoter prior to induction, the lac I repressor gene has been included in the vector and is overexpressed in plasmid-bearing cells.

For convenient cloning and subsequent purification of the protein of interest the pEG-His1 vector contains the following additional features:

- A Multiple Cloning Site (MCS) that allows flexible and easy cloning of the gene of interest
- A start codon, provided by an NdeI site
- Stop codons in all three reading frames downstream of the MCS
- An RGS motif and 6xHis-tag for C-terminal tagging of the protein of interest. This allows easy detection of the fusion protein with commercially available antibodies (# HIST01).
- The 6xHis-tag allows in addition efficient and easy purification of the protein of interest using Immobilized Metal Ion Affinity Chromatography (IMAC), e.g., MoBiTec Ni-IDA Columns (# PR-HTK004) or Ni-NTA Columns.

MoBiTec GmbH, Germany Phone: +49 551 70722 0 Fax: +49 551 70722 22 E-Mail: info@mobitec.com www.mobitec.com

Vector Map and Sequence Details



pEG-His1 5'primer

GATAATGTTTTTTGCGCCGACATCATAACGGTTCTGGCAAATATTCTGAAATGAGCTGT

TGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACA

P_{tac} lacO

CAGGAAACAGTACAT**ATG**GATCCCCGGAATTCCCGGGTCGACTCGAGAGATCTAAGC

start MCS

TTAGAGGATCTCACCATCACCATCACCATGCGGCCGCATCGTGACTGACTGACGATCT

R G S 6xHis 3 x stop

GCCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGAC

pEG-His1 3' primer

Fig.1: Map and Sequence Details of pEG-His1
The whole sequence of pEG-His1 is available at http://www.mobitec.com

Standard Expression Protocol

- Use a single colony to inoculate 2-10 ml of LB medium with 100 µg/ml ampicillin and grow overnight at 37 °C and 200 rpm.
- Dilute the overnight culture 1:10 with fresh medium. For potentially toxic proteins glucose should be added to a final concentration of 2% to increase binding of the LacI repressor protein.
- Grow the diluted culture at 37 °C and 200 rpm for 1-2 hours until the OD_{600} reaches 0.6-0.8.
- Induce expression by adding IPTG to a final concentration of 1 mM.
- Grow the culture for additional 1-6 hours at 37 °C. If required, try lower growing temperatures (e.g., 30 °C or 25 °C) to increase the solubility of the protein of interest.
- Harvest the cells by centrifugation and analyze expression, e.g., by SDS-PAGE and/or Western blot. Recombinant His-tagged fusion proteins may be purified by Ni-IDA (# PR-HTK004) or Ni-NTA affinity chromatography from cleared cell lysates.

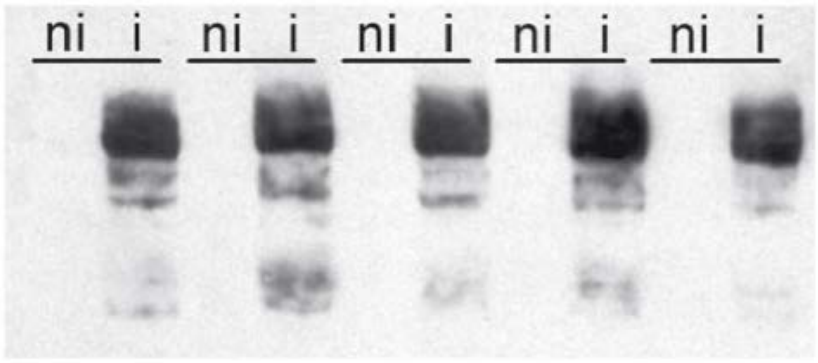


Fig 2: Western blot of whole cell lysates from five *E. coli* clones expressing the toxic protein EBNA2 from Epstein-Barr virus.
ni: not induced, i: induced

Quality Warranty

DNA concentration and purity was checked by UV spectrophotometry. All restriction sites of the MCS and the functional elements Ptac, lacO, RGS-, and 6xHis-tag were checked by sequencing.

Order Information, Shipping, and Storage

Order#	Product	Amount
PEG01	pEG-His1, lyophilized DNA	5 µg
	The vector comes with 500 pmol of 5' and 3' sequencing primers each.	
shipped at room temperature (RT); store at 4 °C Once the DNA has been dissolved in sterile water or buffer we recommend storage at -20 °C.		

Related Products

Order#	Product	Amount
HIST01	anti-His-Tag antibody	100 µl
PR-HTK004	MoBiTec Ni-IDA Columns	4 columns
J106-1KGAM	LB Broth Miller (Luria-Bertani) TC grade	1 kg
0339-25GAM	Ampicillin sodium salt, ultra pure grade	25 g
0487-1GAM	IPTG (Isopropyl-Beta-D-thiogalactoside) ultra pure grade	1 g
E708-1MLAM	IPTG (Isopropyl-Beta-D-thiogalactoside) 20 mg / ml, ultra pure grade	1 ml

Contact and Support

MoBiTec GmbH ● Lotzestrasse 22a ● D-37083 Goettingen ● Germany

Customer Service – General inquiries & orders
phone: +49 (0)551 707 22 0
fax: +49 (0)551 707 22 22
e-mail: order@mobitec.com

Technical Service – Product information
phone: +49 (0)551 707 22 70
fax: +49 (0)551 707 22 77
e-mail: info@mobitec.com

MoBiTec in your area: Find your local distributor at www.mobitec.com