Product Information Sheet

Order: # PEG01

pEG-His 1 vector

SUMMARY

shipped at RT; store at 4 °C

Product Description and Application

- inserts can be expressed as C-terminal tagged 6xHis fusion proteins for efficient and easy one-step
- an RGS motive and the proximate His tag allow detection and/or immunoprecipitation of the expressed protein with commercial anti-RGS and anti-His antibodies (available at MoBiTec, #HIST01)
- optimized promoter guarantees excellent expression levels
- very tight expression control due to overexpression of the LacI repressor
- a convenient MCS allows flexible and easy cloning of the insert
- start codon is provided by an NdeI site

The pEG-His1 vector was constructed for the expression of toxic gene products in *E. coli*. The tac promoter allows highly efficient gene expression after induction with 1 - 5 mM IPTG. To obtain ist exceptional tightness prior induction with IPTG, the LacI repressor gene has been included in the vector and is overexpressed in plasmid bearing cells, thus repressing the tac promoter. Recombinant fusion proteins with a 6xHis tag can be easily and selectively purified using Ni-NTA (nickel-nitrilotriacetic acid) technology*.

*Ni-NTA resin can be obtained from QIAGEN and is exclusively manufactured by QIAGEN under a license from Hoffmann-LaRoche Inc., Nutley, NJ and/or Hoffmann-LaRoche Ltd., Basel, Switzerland. It is provided only for use in research. Information about licenses for commercial use is available from QIAGEN GmbH, Max-Volmer-Strasse 4, D-40724 Hilden, Germany.

Standard Expression Protocol

- Use a single colony to inoculate 2 - 100 ml of LB_{amp} and grow overnight at 37 °C.
- Dilute the overnight culture 1:10 with fresh medium (for potential toxic proteins you should add glucose to a final concentration of 2% to increase LacI binding).
- Grow the cultures at 37 °C 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 (25 °C).
- Harvest the cells by centrifugation.

Analyse expression by SDS-PAGE and/or Western blot.
Isolate recombinant His-tagged fusion proteins by Ni-NTA affinity chromatography following the instructions of the manufacturer.

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Revised August 2010

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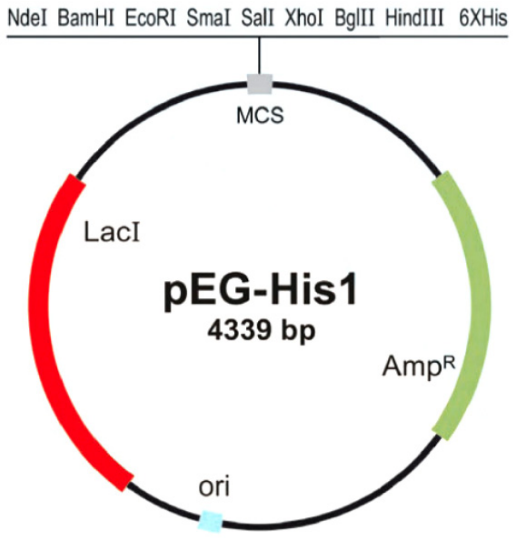
Multiple Cloning Site & Vector Map

Cloning map of pEG-His1 (4339 bp).

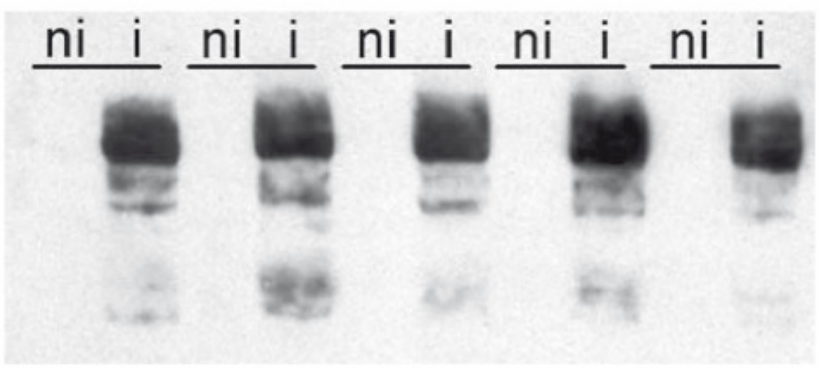


Plasmid map of vector pEG-His1.

Map and multiple cloning site (MCS) of vector pEG-His1

Amp^R: ampicillin resistance,
ori: origin,
LacI: LacI repressor gene.

Sequence and restriction sites of the MCS is listed above.



Western blot of whole cell lysates from five *E. coli* clones expressing the toxic protein EBNA2.

ni: not induced
i: induced

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DNA SEQUENCE

The DNA sequence of pEG-His1 is available for download on our web site (www.mobitec.com).

Order Information, Shipping and Storage

Order#	Product	Quantity
PEG01	pEG-His 1 vector	5 µg
shipped at RT; store at 4 °C		

The vector comes with 500 pmol of 5' and 3' sequencing primers each.

Contact and Support

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