

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.

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 OD₆₀₀ 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.

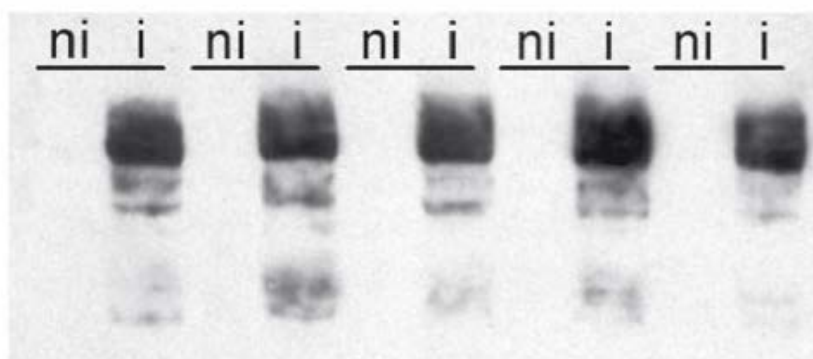


Fig 2: Western blot of whole cell lysates from five *E. coli* clones expressing the toxic protein EBNA2 from Epstein-Barr virus.
ni: not induced, i: induced

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. | | |

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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 |