Certificate of Analysis

pET6xHN Expression Vector Set

Catalog No.	Amount	Lot Number
631432 (Not sold separately) Sold as a part of 631430 & 631431	Each	Specified on product label.

Description

The pET6xHN Expression Vector Set allows you to express your protein of interest with an N- or C-terminal 6xHN tag in *E. coli*. These IPTG-inducible, pET-based vectors contain a T7/*lac* promoter for high-level expression of his-tagged proteins, which can be easily prepped for exceptional purity with our TALON® cobalt resins, or for standard purity and high yield with our high-capacity His60 nickel resins. The system also includes a control vector that expresses an N-terminal, 6xHN-tagged GFPuv fusion protein.

Package Contents

- 20 µl pET6xHN-N Vector (500 ng/µl)
- 20 µl pET6xHN-C Vector (500 ng/µl)
- 10 µl pET6xHN-GFPuv Vector (500 ng/µl)

Storage Conditions

- Store at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Expiration Date

- Specified on product label.

Shipping Conditions

- Dry ice

Product Documents

Documents for our products are available for download at **takarabio.com/manuals**
The following documents apply to this product:

- pET Express & Purify Kits User Manual

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(041323)

pET6xHN-N, pET6xHN-C, and pET6xHN-GFPuv Vector Information

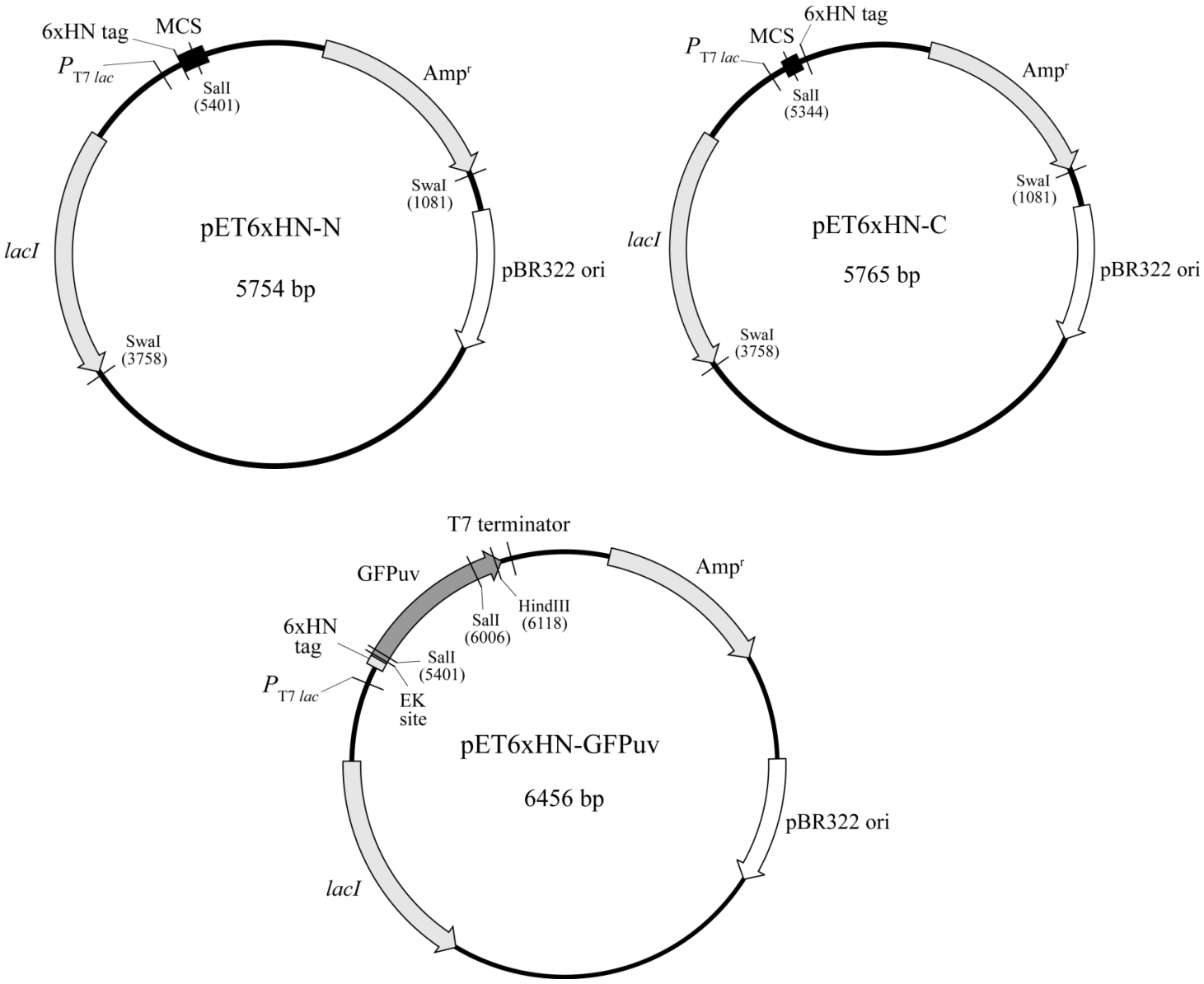

Figure 1. pET6xHN-N, pET6xHN-C, and pET6xHN-GFPuv Vector Maps.

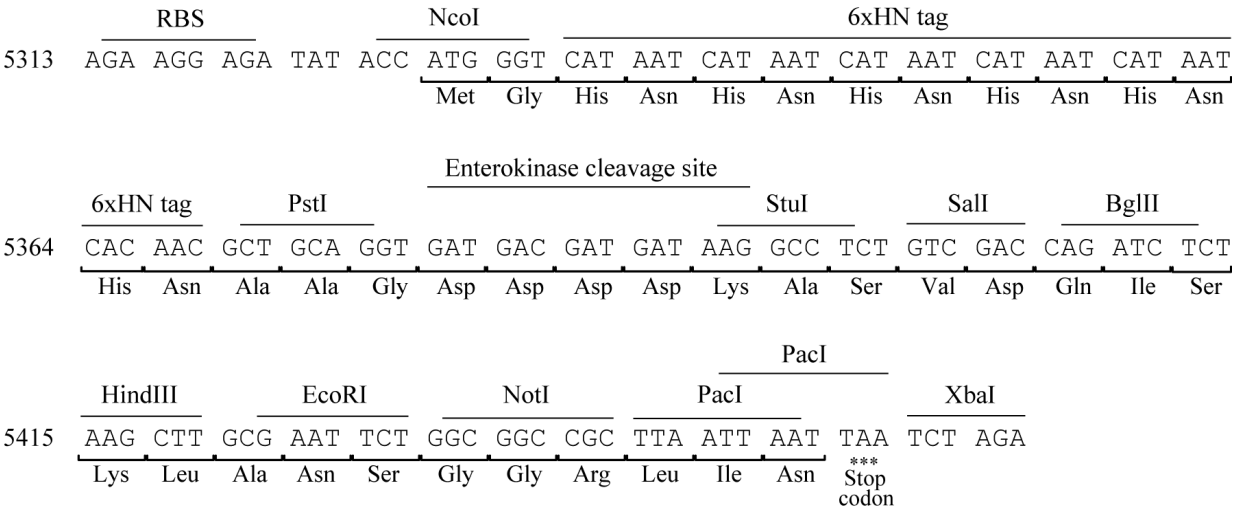

Figure 2. pET6xHN-N multiple cloning site (MCS). The pET6xHN-N vector allows you to add an N-terminal 6xHN tag to your protein of interest.

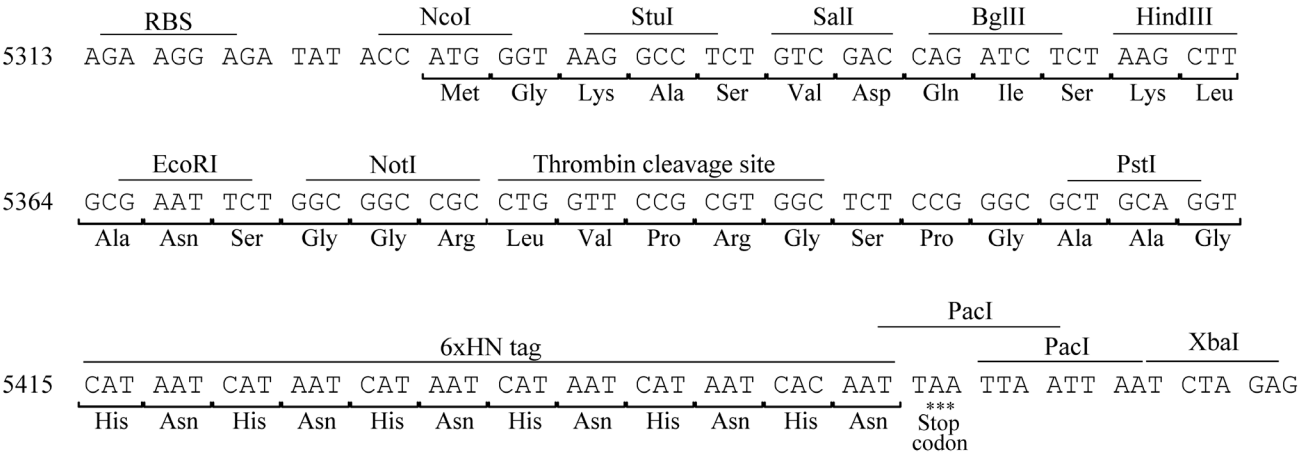

Figure 3. pET6xHN-C multiple cloning site (MCS). The pET6xHN-C vector allows you to add a C-terminal 6xHN tag to your protein of interest.

Description

The vector set contains tightly regulated, yet highly inducible bacterial expression vectors that allow you to express your protein of interest with an N- or C-terminal his tag in *E. coli*. The vectors are based on the pET system vectors developed by William Studier and colleagues at Brookhaven National Laboratories (Dubendorf and Studier 1991; Rosenberg and Studier 1987; Studier and Moffatt 1986; Studier et al. 1990). Derived from pET11 (Dubendorf and Studier 1991), the vectors contain a T7 *lac* hybrid promoter ($P_{T7\ lac}$), which combines the strong T7 promoter with the *lac* operator. Basal expression of the protein of interest is repressed by the Lac repressor (*lacI*), which binds to the *lac* operator, preventing expression from the promoter in the absence of IPTG. High-level, IPTG-inducible expression of the protein of interest is driven by the T7 promoter in the presence of T7 RNA polymerase.

Each vector encodes a 6xHN tag composed of 6 repeating His-Asn subunits, $(His\text{-}Asn)_6$, and either an enterokinase or thrombin cleavage site for subsequent his tag removal. The vectors also contain an ampicillin resistance gene (Amp^r) and a pBR322 origin of replication, which maintains each vector at a low copy number to further reduce basal levels of the protein of interest.

pET6xHN-GFPuv is a control vector that encodes a GFPuv fusion protein containing an N-terminal 6xHN tag and an enterokinase cleavage site. GFPuv is a green fluorescent protein variant optimized for maximal fluorescence when excited by UV light. The vector allows the use of GFPuv fluorescence (excitation and emission maxima at 395 nm and 509 nm, respectively) to monitor protein expression and purification.

Location of Features

pET6xHN-N Vector

- Amp^r (ampicillin resistance gene; beta-lactamase): 210–1067
- pBR322 origin of replication: 1241–1855
- *lacI* (Lac repressor): 3775–4854 (complementary)
- $P_{T7\ lac}$ (T7 *lac* hybrid promoter):
  - T7 promoter: 5241–5257
  - *lac* operator: 5260–5284 (complementary)
- RBS (ribosomal binding site): 5314–5320
- 6xHN tag ([His-Asn]$_6$): 5334–5369
- MCS (multiple cloning site): 5371–5456
- Enterokinase cleavage site: 5379–5393
- T7 terminator: 5495–5541

pET6xHN-C Vector

- Amp^r (ampicillin resistance gene; beta-lactamase): 210–1067
- pBR322 origin of replication: 1241–1855
- *lacI* (Lac repressor): 3775–4854 (complementary)
- $P_{T7\ lac}$ (T7 *lac* hybrid promoter):
  - T7 promoter: 5241–5257
  - *lac* operator: 5260–5284 (complementary)
- RBS (ribosomal binding site): 5314–5320
- MCS (multiple cloning site): 5326–5412
- Thrombin cleavage site: 5382–5399
- 6xHN tag ([His-Asn]$_6$): 5415–5450
- T7 terminator: 5506–5552

pET6xHN-GFPuv Vector

- Amp^r (ampicillin resistance gene; beta-lactamase): 210–1067
- pBR322 origin of replication: 1242–1855
- *lacI* (Lac repressor): 3775–4854 (complementary)
- $P_{T7\ lac}$ (T7 *lac* hybrid promoter):
  - T7 promoter: 5241–5257
  - *lac* operator: 5260–5284 (complementary)
- RBS (ribosomal binding site): 5314–5320
- 6xHN tag ([His-Asn]$_6$): 5334–5369
- Enterokinase cleavage site: 5379–5393
- GFPuv: 5406–6116
- T7 terminator: 6197–6243

Additional Information

Subclone your gene of interest into the MCS. Depending on the restriction sites chosen, your gene of interest can be expressed with or without a 6xHN tag, and with or without an enterokinase cleavage site. Alternatively, you may include any tag of your choice simply by incorporating the tag's sequence into your PCR primers. See the pET Express & Purify Kits User Manual for more information.

In both the pET6xHN-N and the pET6xHN-C vectors, the MCS contains restriction sites for StuI, SalI, BglII, HindIII, EcoRI and NotI in the same reading frame. If these sites are used for cloning, the same insert can be cloned into both vectors in parallel, which is convenient for comparing expression and purification of N-terminally-tagged versus C-terminally-tagged proteins. For convenient cloning using the SalI and HindIII sites, prelinearized versions of both the pET6xHN-N and pET6xHN-C vectors are included in our pET Express & Purify Kit—His60 (In-Fusion® Ready) and pET Express & Purify Kit—HisTALON™ (In-Fusion Ready) systems (Cat. Nos. 631428 & 631429, respectively), which also include our In-Fusion HD Enzyme Premix.

NOTES:

- The MCS is designed with overlapping PacI sites at the 3′ end. This ensures that all three reading frames contain a stop codon. If the PacI site is used for cloning, only one of the sites in the vector will be cut. Thus, be sure that your intended stop codon is found in the first PacI site. That way, the stop codon will be in frame regardless of which PacI site in the vector is digested.
- The XbaI site is downstream of the PacI sites and is therefore not followed by stop codons. If you use the XbaI site for cloning, be sure that your insert contains its own stop codon.

Exceptionally pure his-tagged proteins can be obtained with our TALON Co resins (Cat. Nos. 635501–635504, 635506, 635507, 635509 & 635510) and columns (Cat. Nos. 635601–635603 & 635606). For routine use, we have a variety of high-capacity His60 Ni resins available (Cat. Nos. 635659–635664).

Propagation in *E. coli*

- Suitable host strains for manipulation and propagation: Stellar™ Competent Cells
- Suitable host strains for protein expression: BL21 (DE3) and other DE3 lysogens.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pBR322
- Copy number: low

Excitation and Emission of GVPuv

- Excitation: 395 nm
- Emission: 509 nm

References

Dubendorf, J. W. & Studier, F. W. Controlling basal expression in an inducible T7 expression system by blocking the target T7 promoter with lac repressor. *J. Mol. Biol.* **219,** 45–59 (1991).

Rosenberg, A. H. & Studier, F. W. T7 RNA polymerase can direct expression of influenza virus cap-binding protein (PB2) in Escherichia coli. *Gene* **59,** 191–200 (1987).

Studier, F. W. & Moffatt, B. A. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189,** 113–130 (1986).

Studier, F. W., Rosenberg, A. H., Dunn, J. J. & Dubendorff, J. W. Use of T7 RNA polymerase to direct expression of cloned genes. in *Methods Enzymol.* **185,** 60–89 (Elsevier, 1990).

Quality Control Data

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Vector	Enzymes	Fragments
pET6xHN-N	SalI	5.8 kb
	SwaI	2.7 & 3.1 kb
pET6xHN-C	SalI	5.8 kb
	SwaI	2.7 & 3.1 kb
pET6xHN-GFPuv	HindIII	6.5 kb
	SalI	0.6 & 5.9 kb

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280}: 1.8–2.0

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

Notice to Purchaser

pET6xHN Expression Vector Set

CATALOG NO.

631432

NOTICE TO PURCHASER:

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

Your use of this product is also subject to compliance with the licensing requirements, listed below if applicable, and described on the product´s web page at http://www.takarabio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

TRADEMARKS:

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