Certificate of Analysis

pET6xHN Expression Vector Set (In-Fusion® Ready)

Catalog No.	Amount	Lot Number
631433 (Not sold separately) Sold as a part of 631428 & 631429	Each	Specified on product label.

Description

The pET6xHN Expression Vector Set (In-Fusion Ready) allows you to use In-Fusion cloning technology to express your protein of interest with an N- or C-terminal 6xHN tag in *E. coli*. The vector set contains IPTG-inducible, pET-based vectors that have been prelinearized to allow In-Fusion cloning with the included In-Fusion HD Enzyme Premix. Each vector contains a T7/*lac* promoter for high-level expression of his-tagged proteins, which can be easily prepped for exceptional purity with our TALON® cobalt (Co) resins, or for standard purity and high yield with our high-capacity His60 nickel resins. The vector set also includes a control vector that expresses an N-terminal, 6xHN-tagged GFPuv fusion protein.

Package Contents

- 15 µl pET6xHN-N Vector (In-Fusion Ready) [100 ng/µl]
- 15 µl pET6xHN-C Vector (In-Fusion Ready) [100 ng/µl]
- 10 µl pET6xHN-GFPuv Vector [500 ng/µl]
- 15 µl 1.1 kb LacZ-RK Control Insert [25 ng/µl]
- 20 µl 5X In-Fusion HD Enzyme Premix

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

Takara Bio USA, Inc.
2560 Orchard Parkway, San Jose, CA 95131, USA
U.S. Technical Support: technical_support@takarabio.com

United States/Canada 800.662.2566
Asia Pacific +1.650.919.7300
Europe +33.(0)1.3904.6880
Japan +81.(0)77.565.6999

(042623)

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

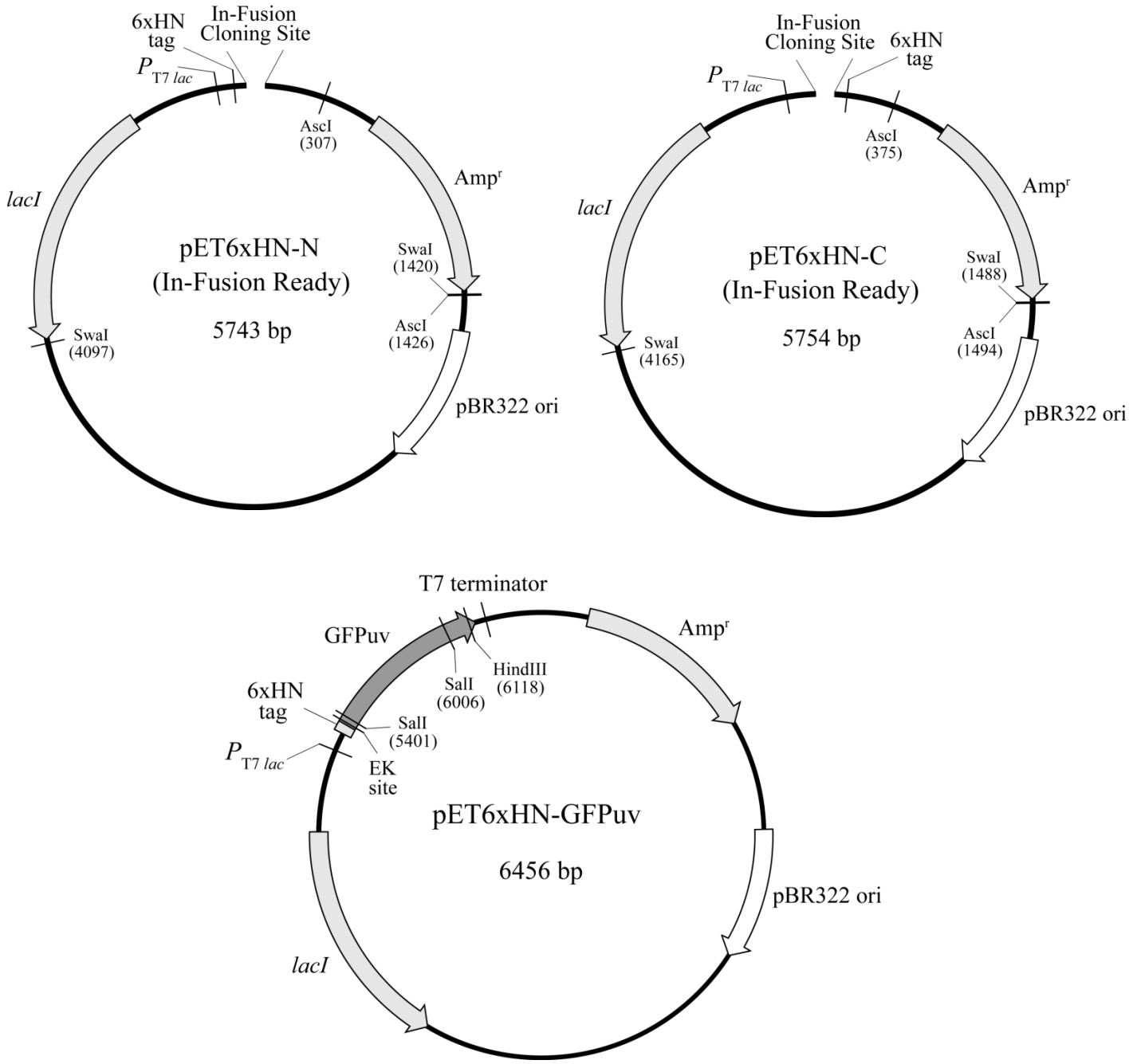

Figure 1. pET6xHN-N (In-Fusion Ready), pET6xHN-C (In-Fusion Ready), and pET6xHN-GFPuv Vector Maps.

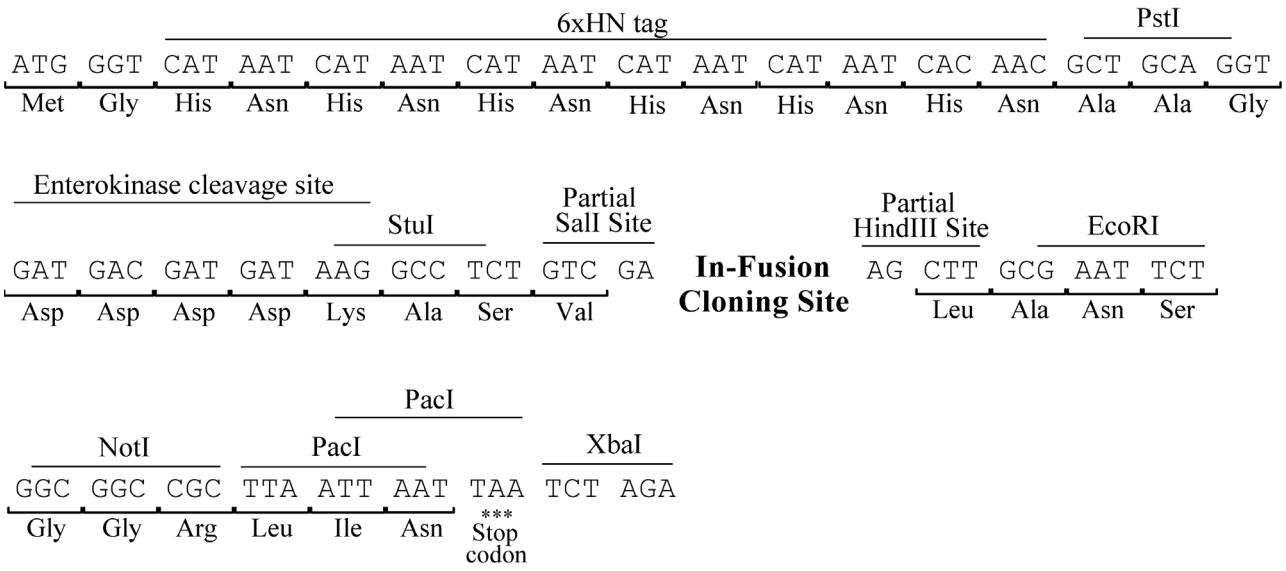

Figure 2. pET6xHN-N (In-Fusion Ready) multiple cloning site (MCS). The pET6xHN-N (In-Fusion Ready) vector has been pre-linearized with SalI and HindIII, allowing you to use In-Fusion technology to add an N-terminal 6xHN tag to your protein of interest.

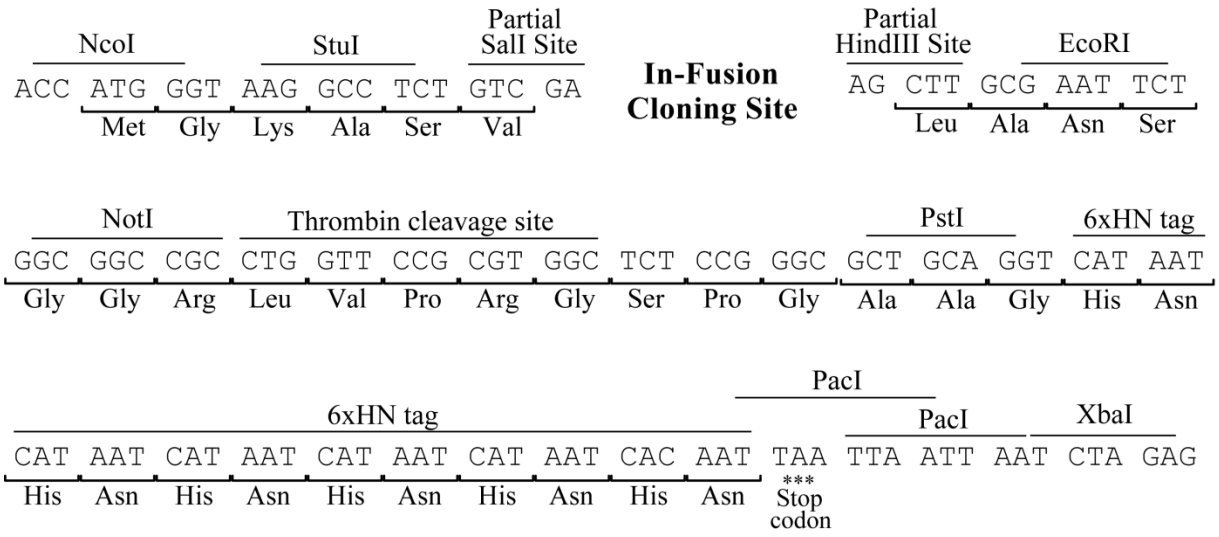

Figure 3. pET6xHN-C (In-Fusion Ready) multiple cloning site (MCS). The pET6xHN-C (In-Fusion Ready) vector has been pre-linearized with SalI and HindIII, allowing you to use In-Fusion technology to add a C-terminal 6xHN tag to your protein of interest.

Description

The vector set contains tightly regulated, highly inducible, bacterial expression vectors (pET6xHN-N and pET6xHN-C) that allow you to express your protein of interest with an N- or C-terminal his tag in *E. coli*. The vectors have been prelinearized with SalI and HindIII for easy In-Fusion cloning of PCR products in-frame with the N- or C-terminal his tag and enterokinase or thrombin cleavage sites. 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 non-linearized 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

- T7 terminator: 80–126
- Amp^r (ampicillin resistance gene; beta-lactamase): 549–1406
- pBR322 origin of replication: 1580–2194
- *lacI* (Lac repressor): 4114–5193 (complementary)
- $P_{T7\ lac}$ (T7 *lac* hybrid promoter):
  - T7 promoter: 5580–5596
  - *lac* operator: 5599–5623 (complementary)
- RBS (ribosomal binding site): 5653–5659
- 6xHN tag ($[His\text{-}Asn]_6$): 5673–5708
- Enterokinase cleavage site: 5718–5732

pET6xHN-C Vector

- Thrombin cleavage site: 24–41
- 6xHN tag ($[His\text{-}Asn]_6$): 57–92
- T7 terminator: 148–194
- Amp^r (ampicillin resistance gene; beta-lactamase): 617–1474
- pBR322 origin of replication: 1648–2262
- *lacI* (Lac repressor): 4182–5261 (complementary)
- $P_{T7\ lac}$ (T7 *lac* hybrid promoter):
  - T7 promoter: 5648–5664
  - *lac* operator: 5667–5691 (complementary)
- RBS (ribosomal binding site): 5721–5727

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

The In-Fusion cloning sites in pET6xHN-N and pET6xHN-C were created by digesting each vector with SalI and HindIII. As a result, the ends of each vector contain partial SalI and HindIII sites. To maintain the correct reading frame, the nucleotides missing from these partial SalI and HindIII sites should be incorporated into your In-Fusion primers. If you would rather not recreate these restriction sites, be certain not to incorporate the missing nucleotides in your In-Fusion primers.

To express your gene of interest without a 6xHN tag and enterokinase cleavage site, do not use your In-Fusion primers to restore the reading frame. If you want to include a tag of your choice, simply incorporate the tag's sequence into your PCR primers. If you wish to clone your gene of interest into other restriction sites in the MCS, we recommend using the circular versions of these vectors (Takara Bio, Cat. Nos. 631430 & 631431).

Exceptionally pure his-tagged proteins can be obtained with our TALON Co resins (Takara Bio, Cat. Nos. 635501–635504, 635506, 635507, 635509 & 635510) and columns (Takara Bio, Cat. Nos. 635601–635603 & 635606). For routine use, we have a variety of high-capacity His60 Ni resins available (Takara Bio, 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. *Methods Enzymol.* **185,** 60–89 (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-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

Functional Assay

In-Fusion HD Enzyme Premix was used to clone 50 ng of the 1.1 kb LacZ-RK Control Insert into both the pET6xHN-N (In-Fusion Ready) vector and the pET6xHN-C (In-Fusion Ready) vector (150 ng each) as described in the User Manual, incubating for 15 min at 50°C. A 2.5 µl aliquot of each mixture was then used to transform Stellar Competent Cells (5 x 10^8 cfu/µg). After 1 hr of growth in 450 µl of SOC Medium, 50 µl of a 1:10 dilution of each transformation mix was plated onto an LB Miller/Amp 100/X-gal IPTG plate. Plates were scored for blue and white colonies after 16–19 hrs.

Negative control cloning reactions lacking the 1.1 kb Control Insert were performed using 150 ng of each vector as described in the User Manual, incubating for 15 min at 50°C. A 2.5 µl aliquot of each negative control reaction was used to transform Stellar Competent Cells (5 x 10^8 cfu/µg). After 1 hr of growth in 450 µl of SOC Medium, each transformation mix was plated onto an LB Miller/Amp 100/X-gal IPTG plate.

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 (In-Fusion® Ready)

CATALOG NO.

631433

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:

Takara Bio USA, Inc.
2560 Orchard Parkway, San Jose, CA 95131, USA
U.S. Technical Support: technical_support@takarabio.com

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.565.6999

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