Cat. # HB121-HB123

For Research Use

TaKaRa

Brevibacillus Expression System His-Tag Fusion Expression Vectors

pNC-HisT DNA (Cat. #HB121)
pNC-HisF DNA (Cat. #HB122)
pNC-HisE DNA (Cat. #HB123)

Product Manual

v202104

Table of Contents

I. Description

The *Brevibacillus* Expression System is a high-yield, secretory protein production system. The system uses *Brevibacillus choshinensis*, a gram-positive bacterium that is characterized by its ability to secrete large amounts of protein[1)].
This characteristic has been successfully used for the production of a large number of heterologous proteins. This system has the following features:

- Extracellular secretion of large volumes of protein
- Almost no detectable protease activity
- Production of active protein
- Simple procedures for genetic manipulation and culturing
- A safe host bacteria

Examples of protein production using this system are shown in Table 1. High expression of biologically active protein (enzymes, antigens, and cytokines) was obtained, regardless of gene origin (bacterial, archaea, or eukaryotic). In particular, eukaryotic secretory proteins often contain disulfide (S-S) bonds and are generally difficult to produce in prokaryotic expression systems. The *B. choshinensis* Host-Vector System can produce high yields of secretory proteins, including those with disulfide bonds.

Table 1. Examples of Successful Production of Heterologous Proteins with the *B. choshinensis* Host-Vector System

Protein	Origin	Production (g/L)	Reference
Enzymes			
α-Amylase	*B. licheniformis*	3.7	
Sphingomyelinase	*B. cereus*	3.0	
Xylanase	*B. halodurans*	0.2	
CGTase	*B. macerans*	1.5	2)
Chitosanase	*B. circulans*	1.4	
Hyperthermostable protease	*A. pernix*	0.1	
Hyperthermostable nuclease	*P. horikoshii*	0.7	
PDI	Human	1.0	3)
Antigen			
Surface antigen	*E. rhusiopathiae*	0.9	
Surface antigen	*T. pallidum*	0.8	
Cytokine			
EGF	Human	1.5	4)
NGF	Mouse	0.2	
IFN-γ	Chicken	0.5	5)
TNF-α	Bovine	0.4	
GM-CSF	Bovine	0.2	
GH	Flounder	0.2	

The host strain has a high transformation efficiency and is amenable to genetic manipulation. By using a shuttle vector, expression vectors can be constructed in *E. coli* and be used to transform *B. choshinensis.*
Culturing for production of the target protein uses one of two types of media (refer to V-9. Medium components). The production process is simple: cells are cultured in culture tubes or flasks on a shaker, and the culture supernatant is harvested by centrifugation. There is no need to disrupt bacterial cells; centrifugation of the cell culture will remove cells and yield a clear supernatant containing the target protein, which can be used for subsequent purification procedures.

pNC-HisT DNA, pNC-HisF DNA, and pNC-HisE DNA are expression vectors, allowing secretion of the target protein as a his-tagged fusion protein using this system.

II. Components

pNC-HisT DNA (Cat. #HB121)	10 μg (0.2 μg/μl)
pNC-HisF DNA (Cat. #HB122)	10 μg (0.2 μg/μl)
pNC-HisE DNA (Cat. #HB123)	10 μg (0.2 μg/μl)

【Form】 10 mM Tris-HCl, pH 8.0
1 mM EDTA

III. Storage

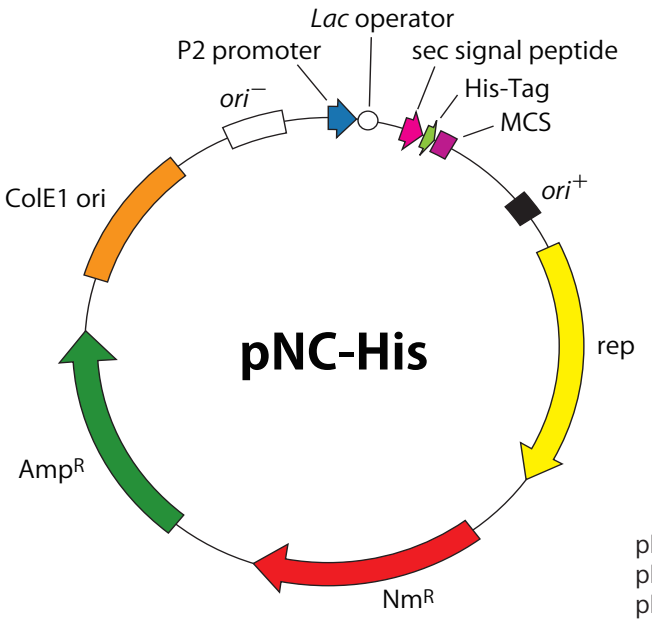
-20℃
＊ 2 years from date of receipt under proper storage conditions.

IV. Overview of *Brevibacillus* Expression System His-Tag Fusion Expression Vector

The workflow to produce the target protein using this product is described as below.

IV-1. Selection of Expression Vectors

pNC-HisT DNA, pNC-HisF DNA, and pNC-HisE DNA are shuttle vectors between *Brevibacillus* and *E. coli* derived from pNCMO2 DNA (Cat. #HB112), a secretory expression vector. These vectors are constructed with insertion of a his-tag sequence (6 x His) and a protease recognition sequence for tag cleavage downstream of the secretion signal. As with pNCMO2, the expression plasmids are constructed in *E. coli* and then are used to transform *Brevibacillus* for protein expression.
The his-tag sequence is followed by a thrombin sequence in pNC-HisT DNA, a factor Xa sequence in pNC-HisF DNA, or an enterokinase sequence in pNC-HisE DNA.
Like pNCMO2, these vectors use the P2 promoter of the host cell wall protein for expression. This P2 promoter is useful for cloning target genes because it is a strong promoter in *Brevibacillus* but is non-functional in *E. coli*, thereby allowing efficient protein production in *Brevibacillus.*
This potent promoter activity may interfere with the growth of transformants. In such a case, use pNY326 DNA, which has weaker promoter activity and can be stably maintained in the host cell. (However, with this vector, there will not be an N-terminal his-tag.)



pNC-HisT DNA: 5,260 bp
pNC-HisF DNA: 5,260 bp
pNC-HisE DNA: 5,263 bp

Figure 1. Vector Map of pNC-His DNA

<Features of pNC-His-Series Vectors>

P2 promoter	Uses part of a 5′ sequence of the cell wall protein (HWP) gene, which exhibits very little activity in *E. coli* but is a potent promoter in *Brevibacillus*
Sec signal peptide	Secretion signal sequence of HWP, modified to increase secretion efficiency.
MCS (multicloning site)	Protease recognition sequence: 9 restriction enzyme cleavage sites
His-Tag + protease recognition sequence	Enterokinase (pNC-HisE) Factor Xa (pNC-HisF) Thrombin (pNC-HisT)
Terminator	A 46-bp nucleotide sequence functioning as a terminator introduced downstream of the multi-cloning site.
Rep	Gene involved in plasmid replication (pUB110 derived)
Ori	Replication origin for replication and maintenance of the plasmid in *Brevibacillus* (pUB110 derived)
Nm^R	Neomycin resistance gene, a selection marker in *Brevibacillus*
ColE1 *ori*	Replication origin for replication and maintenance of the plasmid in *E. coli* (pUC-derived).
Amp^R	Ampicillin resistance gene, selection marker in *E. coli*

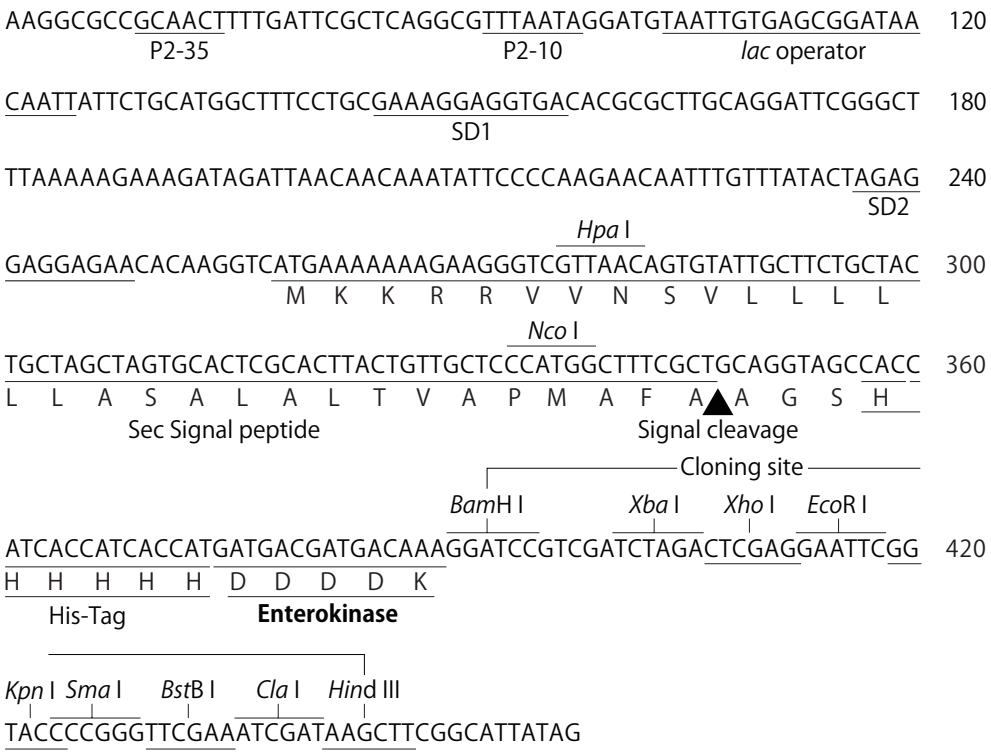
< Cloning sites of pNC-HisT DNA >

```
AAGGCGCCGCAACTTTTGATTCGCTCAGGCGTTTAATAGGATGTAATTGTGAGCGGATAA 120
          P2-35                 P2-10              lac operator
CAATTATTCTGCATGGCTTTCCTGCGAAAGGAGGTGACACGCGCTTGCAGGATTCGGGCT 180
                          SD1
TTAAAAAGAAAGATAGATTAACAACAAATATTCCCCAAGAACAATTTGTTTATACTAGAG 240
                                                      SD2
                                    Hpa I
GAGGAGAACACAAGGTCATGAAAAAAAGAAGGGTCGTTAACAGTGTATTGCTTCTGCTAC 300
                M  K  K  R  R  V  V  N  S  V  L  L  L  L
                                 Nco I
TGCTAGCTAGTGCACTCGCACTTACTGTTGCTCCCATGGCTTTCGCTGCAGGTAGCCACC 360
L  L  A  S  A  L  A  L  T  V  A  P  M  A  F  A▲A  G  S  H
      Sec Signal peptide                  Signal cleavage
                                                  Cloning site
                                 BamH I     Xba I  Xho I  EcoR I  Kpn I
ATCACCATCACCATCTGGTTCCACGTGGATCCGTCGATCTAGACTCGAGGAATTCGGTAC 420
H  H  H  H  H  L  V  P  R  G  S
      His-Tag          Thrombin

 Sma I   BstB I   Cla I   Hind III
CCCGGGTTCGAAATCGATAAGCTTCGGCATTATAG
```

< Cloning sites of pNC-HisF DNA >

```
AAGGCGCCGCAACTTTTGATTCGCTCAGGCGTTTAATAGGATGTAATTGTGAGCGGATAA 120
          P2-35                 P2-10              lac operator
CAATTATTCTGCATGGCTTTCCTGCGAAAGGAGGTGACACGCGCTTGCAGGATTCGGGCT 180
                          SD1
TTAAAAAGAAAGATAGATTAACAACAAATATTCCCCAAGAACAATTTGTTTATACTAGAG 240
                                                      SD2
                                    Hpa I
GAGGAGAACACAAGGTCATGAAAAAAAGAAGGGTCGTTAACAGTGTATTGCTTCTGCTAC 300
                M  K  K  R  R  V  V  N  S  V  L  L  L  L
                                 Nco I
TGCTAGCTAGTGCACTCGCACTTACTGTTGCTCCCATGGCTTTCGCTGCAGGTAGCCACC 360
L  L  A  S  A  L  A  L  T  V  A  P  M  A  F  A▲A  G  S  H
      Sec Signal peptide                  Signal cleavage
                                                  Cloning site
                                 BamH I     Xba I  Xho I  EcoR I  Kpn I
ATCACCATCACCATATCGAAGGTCGTGGATCCGTCGATCTAGACTCGAGGAATTCGGTAC 420
H  H  H  H  H  I  E  G  R
      His-Tag          Factor Xa

 Sma I   BstB I   Cla I   Hind III
CCCGGGTTCGAAATCGATAAGCTTCGGCATTATAG
```

< Cloning sites of pNC-HisE DNA >



IV-2. Cloning into expression vectors

The expression vectors contain a secretion signal from a cell wall protein. These vectors contain a multicloning site downstream of the secretion signal, a his-tag sequence, and a protease recognition site. Using two different restriction enzyme sites on the multicloning site, the target gene DNA can be cloned in the intended direction.

IV-3. Transformation of *Brevibacillus*

Transformation of *Brevibacillus* is achieved by New Tris-PEG (NTP) methods. Selection is based on neomycin resistance. When a shuttle vector is used for subcloning in *E. coli,* ampicillin resistance can be used as the selection marker.

IV-4. Detection of protein production and scale-up

Select transformants containing the expression plasmid for the target protein and culture in the specified liquid medium on a shaker for 48 - 64 hours to obtain the target protein. Use an aliquot of the culture supernatant for SDS-PAGE analysis or other test to confirm the expression of the target protein. Scale up the culture volume to increase the protein yield. Use a negative control to confirm expression of the target protein. Large scale culturing of *Brevibacillus* is relatively easy.

V. Protocol

V-1. *Brevibacillus* strain

Standard genetic techniques can be used.

V-1-1. Genotype

Because genes relating to sporulation have been disrupted, it is easy to perform sterilization of the strain. Additionally, disruption of intracellular protease gene (*imp*) and extracellular protease gene (*emp*) minimizes degradation of expressed recombinant proteins.

V-1-2. Storage of recombinant *Brevibacillus*

Short-term storage (about 1 week)

1. Pick a single colony and spread on a MTNm plate.
2. Culture overnight at 30℃.
3. Seal the plate and store at room temperature (approximately 20℃).

Note: Never store the plate in a refrigerator (4℃).

Long-term storage (1 month or longer)

1. Pick a single colony, inoculate 2SYNm medium (See V-8. Composition of Medium), and culture overnight with shaking.
2. Transfer to a vial for cryopreservation and add an equal volume of LB medium containing 40% glycerol.
3. Store frozen at -80℃.
4. Thaw each vial immediately before use and inoculate at 0.1 - 1.0% volume of liquid medium.

Note: Do not subject cell stocks to repeated freeze-thaw cycles.

V-2. *E. coli* host

A *lac* operator is inserted in the pNC-His series to weaken the promoter activity in *E. coli*. Hence, a host strain containing an F factor (*lac* I^q), such as JM109, must be used. The genotype of JM109 is shown below for reference.

JM109: *recA1, endA1, gyrA96 thi-1, hsdR17* (r_K^- m_K^+), *e14* $^-$ (*mcrA* $^-$), *supE44, relA1*, △ (*lac-proAB*) /F' [*traD36, proAB* $^+$, *lac* I^q, *lacZ* △ M15]

V-3. Construction of expression vectors using the pNC-His series

Procedures and precautions for the construction of expression vectors using the pNC-His series

E. coli strains containing *lac* I^q and *recA*, such as JM109, are recommended as the host for plasmid construction.

An insert DNA should be cloned in the vector in-frame with, and downstream of the secretion signal and his-tag.

A stop codon must be introduced at the 3' end of the gene.

When expressing a bacterial secretory protein, using the protein's own secretion signal may in some cases provide better results. In such a case, the use of pNY326 vector is required. (This vector does not contain an N-terminal his-tag.)

Note:

< Cloning using the In-Fusion® cloning system >

The In-Fusion HD Cloning Kit (Cat. #639633) offers a simple, convenient protocol for fast directional cloning, even in the absence of an appropriate restriction enzyme site. Follow the protocol for the In-Fusion cloning system.

V-3-1. Cloning the target gene into the pNC-His series

< Gene amplification by PCR >

Design primers to allow insertion of the target gene downstream of the secretion signal. Introduce restriction enzyme sites, appropriate for the expression vector, into 5' and 3' ends of the PCR primers, to provide insert directionality. Then amplify the target gene by PCR using these primers. Select PCR conditions appropriate for each gene and PCR enzyme. Use a high-fidelity PCR enzyme (e.g., PrimeSTAR® Max DNA Polymerase (Cat. #R045A)).

< Ligation with expression plasmids >

Digest the insert and the vector (0.5 - 1.0 μg) with two restriction enzymes. Purify each target fragment by agarose gel electrophoresis.
Perform ligation using 100 ng of each purified DNA with the DNA Ligation Kit <Mighty Mix> (Cat. #6023) or another similar DNA ligation kit.

< Transformation of *E. coli* >

Transform *E. coli* host cells with a high transformation efficiency, such as *E. coli* JM109 Competent Cells (Cat. #9052) or *E. coli* JM109 Electro-Cells (Cat. #9022), with an aliquot of the mixture.

V-3-2. Analysis of *E. coli* transformants

Plate 100 - 200 μl of the *E. coli* transformation mixture onto LB plates containing 50 - 100 μg/ml of ampicillin. Incubate at 37℃ for 15 - 18 hours.
Select 10 - 20 ampicillin-resistant colonies and inoculate each in 2 ml of LB medium (containing 50 - 100 μg/ml of ampicillin). Incubate at 37℃ for 15 - 18 hours.
Collect cells by centrifugation. Purify plasmids using a commercial kit. In general, 1.5 - 3 μg of plasmid DNA can be recovered.
Digest an appropriate amount of plasmid DNA with restriction enzymes to confirm the presence of the insert DNA by agarose gel electrophoresis.
In addition, perform sequencing to make sure the target gene is inserted in the correct direction and reading frame and no sequence errors are present.

V-3-3. Sequencing

For sequencing verification, the following sequencing primers can be used. (pNCMO2 and pNY326 are available in the same primer sequences.)

Forward Sequencing Primer: 5′-CGCTTGCAGGATTCGG-3′
Reverse Sequencing Primer: 5′-CAATGTAATTGTTCCCTACCTGC-3′

V-3-4. Purification of expression vector

Purify the expression vector using a commercial kit from the transformant culture.

V-4. Transformation of *Brevibacillus*

Use *Brevibacillus* Competent Cells (Cat. #HB116) for transformation.

V-4-1. Preparation of materials

Prepare the following reagents and materials.

- *Brevibacillus* Competent Cells (Cat. #HB116)
  - *Brevibacillus* Competent Cells
  - MT Medium＊1
  - Solution A
  - Solution B
- Plasmid for target gene expression
- MTNm plates＊1
- Culture tubes＊2
- Sterilized microtubes

＊1 Refer to V-9. Medium Components
＊2 e.g., 14-ml round-bottom sterile tube (falcon tube).

V-4-2. NTP Transformation Method

(1) Thaw Solution A, Solution B, and MT medium.
(2) Remove only the number of tubes of *Brevibacillus* Competent Cells needed for transformation from storage, and keep on dry ice/ethanol.
(3) Thaw the *Brevibacillus* Competent Cells quickly (approximately 30 seconds) in a 37℃ water bath.
(4) Centrifuge the cells (12,000 rpm for 30 seconds to 1 minute) to pellet the cells and remove the supernatant with a micropipette.

Perform the following procedures at room temperature.

(5) Mix the plasmid DNA solution (in a volume of 5 μl or less)＊1 with 50 μl of Solution A.
(6) Add all of the DNA solution to the bacterial cell pellet (from step 4) and vortex to completely suspend the bacterial pellet.＊2
(7) Allow to stand for 5 minutes at room temperature.
(8) Add 150 μl of Solution B (PEG solution)＊3 and vortex until the solution is uniform (5 - 10 seconds).
(9) Centrifuge the cells (5,000 rpm for 5 minutes) and remove the supernatant.
(10) Centrifuge briefly (5,000 rpm for 30 seconds) and remove the supernatant completely.
(11) Add 1 ml of MT medium and suspend completely with a micropipette.
(12) Transfer the medium containing the cells into a culture tube, then incubate for 2 hours at 37℃ in an orbital shaker (120 rpm).
(13) Use a sterile inoculating loop to remove a small sample from the culture. Streak on a MTNm plate and culture overnight at 37℃.
(14) Select isolated colonies for plasmid analysis or protein expression.

＊1 Use 10 - 100 ng of the purified plasmid.
＊2 Be sure to suspend well, as incomplete dispersion of cells will decrease the efficiency of transformation.
＊3 Solution B (PEG solution) is highly viscous; use a 1,000 μl micropipette and pipette slowly.

V-5. Expression of target proteins from recombinant *Brevibacillus*

Perform small-scale protein expression with the transformed *Brevibacillus* cells. A standard method for confirming protein expression is given here.

V-5-1. Overview

Protein expression level and colony size of transformants may vary depending on the target protein. Therefore, 6 - 10 colonies (including both large and small colonies) should be randomly selected for expression testing in test tube cultures. If plates stand for multiple days after transformation, protein production may decrease. If this occurs, perform transformation again.

V-5-2. Culture medium

TM medium and 2SY medium are used as the media for expression testing. Examine protein production with both types of media because using a different medium may cause differences in protein expression level.

V-5-3. Protein production (secretory production)

The protocol for expression testing is shown below.

Note: To confirm specific expression of the target protein, also perform the experiment using a negative control clone.

(1) Select colonies and inoculate each colony into two separate 3 ml cultures, one in 2SYNm liquid medium and the other in TMNm liquid medium, in 16 mm culture tubes. Incubate at 30 - 33°C with shaking at 120 rpm under sufficient ventilation for 48 - 64 hours. During the incubation, remove aliquots of the culture every 24 hours to confirm target protein production.
(2) At the end of the incubation, isolate the supernatant by centrifugation (5,000*g* for 5 minutes). Suspend the cell precipitate in an equal volume of PBS.*
(3) Perform SDS-PAGE (CBB staining or Western blotting) or an activity assay on the supernatant and the precipitate fraction.

* For easy preparation, use PBS (Phosphate Buffered Salts) Tablets (Cat. #T900).

V-6. SDS-PAGE analysis

Perform electrophoresis using an SDS-PAGE gel suitable for analyzing the target protein.

V-6-1. Sample preparation

Add 10 μl of 5X SDS-PAGE loading buffer to 40 μl of culture supernatant or cell precipitate.
Mix and heat at 100℃ for 10 minutes to prepare samples for SDS-PAGE.

V-6-2. Control

Use following samples as the control.

a. Molecular size marker protein
b. The target protein standard
c. Sample from the culture of *B. choshinensis* SP3 containing a vector without the target gene (negative control)

V-6-3. Analysis of protein expression

The presence/absence of protein production can be confirmed by comparing the target protein standard and the culture supernatant on SDS-PAGE. When protein detection is difficult due to low expression, low solubility, or masking by the background proteins, protein production should be verified by Western blot analysis with an antibody specific to the target protein, functional evaluation (e.g., specific activity), or protein purification.
When pNC-His series is used as an expression vector, Western blotting with an anti-His-tag antibody can be used to detect the target protein.

V-7. Optimization of protein expression

A number of experiments have shown high-level protein expression that exceeded 1 mg/ml culture. Most target proteins can be produced at 100 μg/ml culture or more. In the case of low or no protein production, please refer to the guidelines below.

V-7-1. Low expression

a. Try to use other vectors such as pNY326 (sold separately) with different levels of promoter activity. The vectors of the pNC-His series have higher promoter activity and produce greater amounts of some proteins. However, protein production may be increased by improving bacterial growth by using a vector with a lower level of promoter activity such as pNY326.
b. Use a different type of medium. Protein production may vary depending on the medium type.
c. Some proteins may not be suitable for secretory production. Try intracellular expression by using an intracellular expression vector of the pNI series.

V-7-2. No expression

Proceed with the same experiments as V-7-1. "Low expression". If no improvement results, consider the following points:

a. Check the secondary structure of mRNA. The presence of a high-energy palindromic structure may cause translation abnormalities. In such case, it is necessary to insert mutations in the repeat sequence.
b. Inappropriate sequence neighboring the signal cleavage site may affect the secretory process. If additional sequence insertion to the N-terminus of the target protein will not affect the target protein's activity, production may improve by inserting a purification tag or a detection tag, or random sequence.

V-8. Protein purification

Protein purification methods vary according to the type of target protein. If the target protein is successfully secreted in the medium, supernatant containing the target protein can be obtained by centrifugation of the bacterial culture.
If pNC-His series is used, the target protein can be easily purified using TALON® Metal Affinity Resin (Cat. #635501) or another histidine-tagged protein purification resin. Moreover, the his-tag region can be easily removed by treatment with the respective protease (Enterokinase, Factor Xa, or Thrombin) corresponding to each recognition sequence. For purification with his-tagged protein purification resin and protease treatment, refer to the corresponding manual for each product.

Note:
Directly applying 2SY medium to a Ni-chelate column may cause Ni to be removed from the column resin. If 2SY medium is used for culturing the recombinant cells, purification can be successfully performed after dialysis of the culture sample. Dialysis is unnecessary when using Ni Sepharose Fast Flow (GE Healthcare).

V-9. Medium components

・2SY liquid medium

Components	
Glucose*	20.0 g/L
Bacto Soytone	40.0 g/L
BactoYeast Extract	5.0 g/L
$CaCl_2 \cdot 2H_2O$	0.15 g/L
Adjust to pH7.2 with NaOH	

* Sterilize the glucose and $CaCl_2$ separately from the other components. Mix after sterilization.

・2SY Nm liquid medium

Add neomycin solution (stock solution: 50 mg/ml) to 2SY liquid medium to a final concentration of 50 μg/ml.

・TM liquid medium

Components

Glucose*	10.0 g/L
Phytone Peptone	10.0 g/L
35%Ehrlich Bonito extract	5.75 g/L
Yeast extract Blue label	2.0 g/L
$FeSO_4 \cdot 7H_2O$	10 mg/L
$MnSO_4 \cdot 4H_2O$	10 mg/L
$ZnSO_4 \cdot 7H_2O$	1 mg/L
Adjust to pH7.0 with NaOH	

＊ Sterilize glucose and other components separately. Mix after sterilization.

・TMNm medium

Add neomycin solution (stock solution 50 mg/ml) to TM liquid medium to a final concentration of 50 μg/ml.

・MT liquid medium

Add $MgCl_2$ to the TM liquid medium to reach 20 mM.

・MTNm plate

Suspend 7.5 g of agar in 500 ml of MT liquid medium and autoclave. After cooling to approximately 50℃, add neomycin solution (50 mg/ml stock solution) to obtain a final concentration of 50 μg/ml, mix gently, and dispense into plates.

For the components of the 2SY medium, TM medium, and TMNm medium, the following manufacturers are recommended.

Bacto Soytone	(Becton Dickinson, Code. 243620)
Bacto Yeast Extract	(Becton Dickinson, Code. 212750)
Phytone Peptone	(Becton Dickinson, Code.211906)
35%Ehrlich Bonito Extract	(Kyokuto Pharmaceutical, Code 551-01212-5)
Yeast extract Blue label	(Oriental Yeast Co., Ltd.)
Neomycin	(Sigma)

VI. Experimental Example: Expression and Purification of *Bacillus licheniformis* α-amylase (BLA) using pNC-HisT

In an expression experiment in TMNm medium (30℃ for 48 hours) using the pNC-HisT cloned with the BLA gene, SDS-PAGE analysis showed high levels of protein production (approximately 0.2 mg/ml) in the culture supernatant (Figure 2). The his- tagged BLA was purified from the supernatant using TALON Metal Affinity Resin. SDS-PAGE analysis (Figure 3A) showed a high degree of purity and a nearly 100% recovery rate. Then the purified protein was digested by thrombin. A change in the mobility of the protein on SDS-PAGE confirmed the removal of His-Tag region (Figure 3B). This was further verified by Western blots in which the protease-digested proteins did not react with the His-Tag antibody (data not shown).

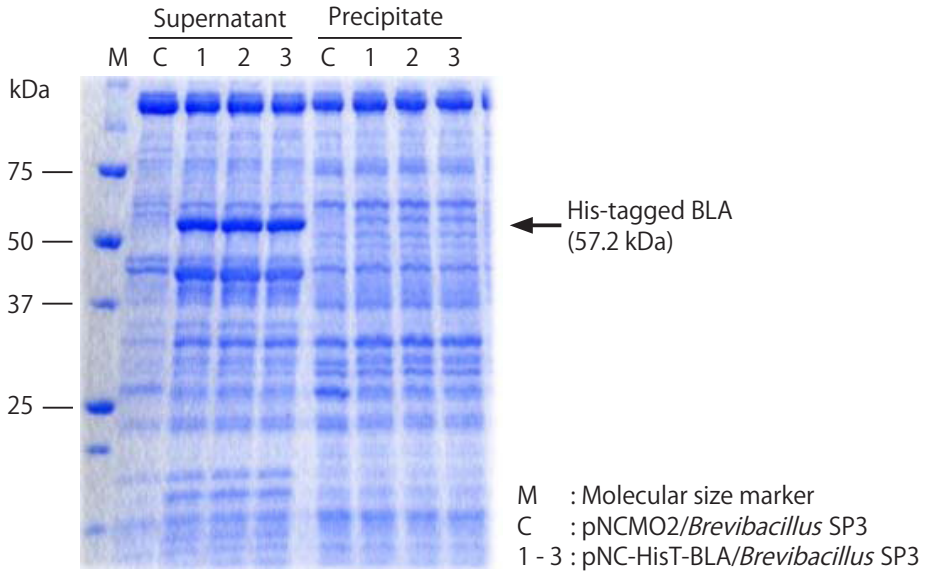
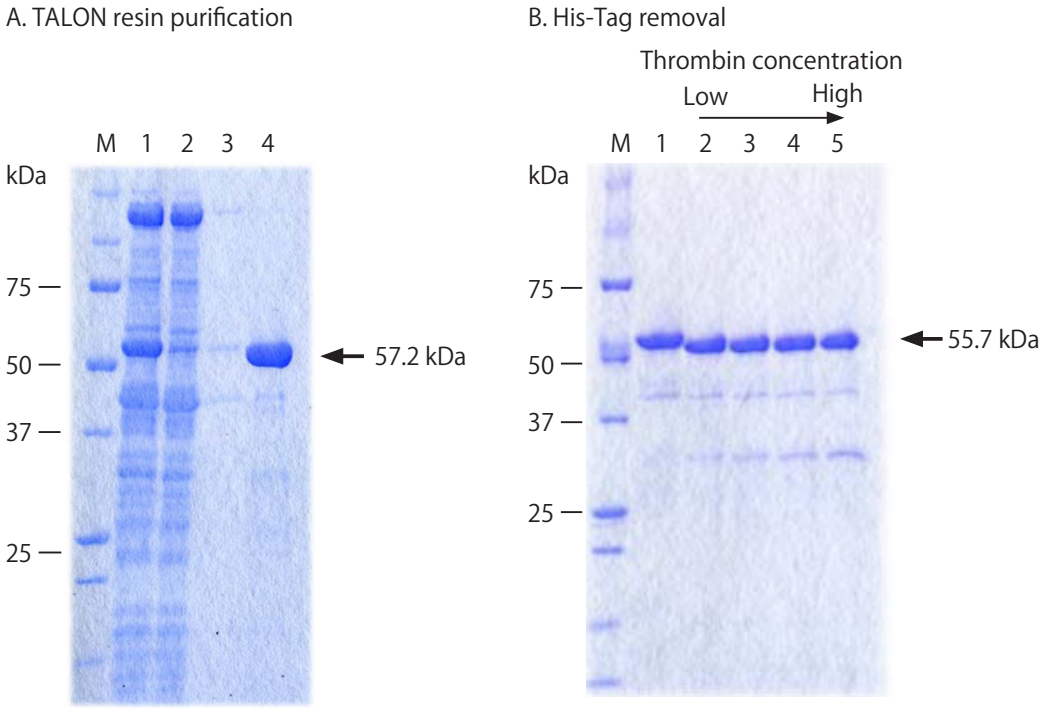


Figure 2. Secretory Expression of α-amylase (BLA) using pNC-HisT



M : Molecular size marker
1 : Culture supernatant
2 : Flow-through
3 : Wash
4 : Elution fraction

M : Molecular size marker
1 : Purified His-tagged BLA
2 - 5 : Thrombin-treated (16 hrs.)

Figure 3. Purification of α-amylase (BLA) produced using pNC-HisT

In expression and purification experiments using pNC-HisF and pNC-HisE with the BLA gene, digestion with Factor Xa or enterokinase produced similar results (date not shown).

VII. Related Products

[*Brevibacillus* Secretory Expression System]
- BIC System (Cat. #HB300)
- pBIC DNA Set (Cat. #HB310)
- *Brevibacillus* Expression System II (Cat. #HB200)
- *Brevibacillus* Competent Cells (Cat. #HB116)
- pNCMO2 DNA (Cat. #HB112)

[Intracellular Expression Vector]
- pNI DNA (Cat. #HB131)
- pNI-His DNA (Cat. #HB132)

[His-tagged Protein Purification]
- HisTALON™ Superflow Cartridge Purification Kit (Cat. #635649)
- HisTALON™ Superflow Cartridge (Cat. #635650)
- HisTALON™ Buffer Set (Cat. #635651)
- TALON® Metal Affinity Resin (Cat. #635501/635502/635503/635504/635652/635653)

[Others]s
- In-Fusion® HD Cloning Plus (Cat. #638909)
- *E. coli* JM109 Competent Cells (Cat. #9052)
- *E. coli* JM109 Electro-Cells (Cat. #9022)
- DNA Ligation Kit <Mighty Mix> (Cat. #6023)
- PrimeSTAR® Max DNA Polymerase (Cat. #R045A)
- PBS (Phosphate Buffered Salts) Tablets (Cat. #T900)

VIII. References

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2) T. Takano, A. Miyauchi, H. Takagi, K. Kadowaki, K. Yamane, and S. Kobayashi. Expression of the Cyclodextrin Glucanotransferase Gene of Bacillus macerans in *Bacillus brevis*. *Biosci Biotech Biochem*. (1992) **56**(5): 808-809.
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