

## PRODUCT INFORMATION

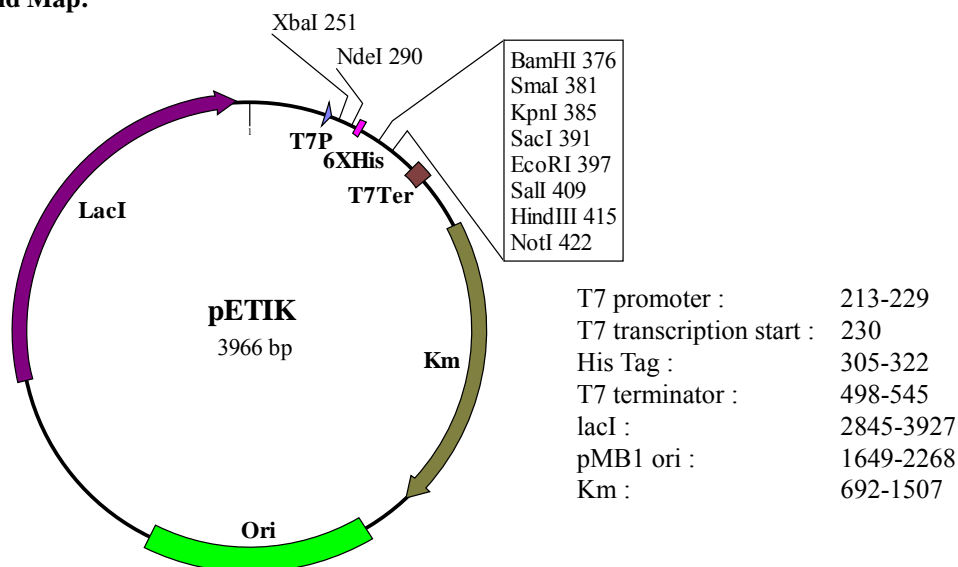
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### GENERAL INFORMATION

- Product Name :** Expression Vector pETIK  
**Code No. :** DV235  
**Size :** 15 µg (lyophilized plasmid contains salt of TE buffer)  
**Storage :** This product is shipped at ambient temperature. Upon receipt, store at - 20 °C  
**Reconstitution :** Resuspend the lyophilized pETIK with 15 µl of sterile water to make 1 µg/µl plasmid in 1 × TE buffer. After reconstitution, store at - 20 °C

**Product Description :** pETIK is a medium copy number, kanamycin resistant, stringent controllable T7 bacterial expression vector. The T7 expression system is one of the strongest expression systems and has been widely used with a coupling of BL21 (DE3) E. coli cell. T7 RNA polymerase gene is integrated in a genome of BL21(DE3) under control of lacUV5 promoter. Upon addition of isopropyl-1-thio-β-D-galactopyranoside (IPTG), T7 RNA polymerase is expressed in the BL21(DE3) cells harboring pETIK vector, and it induces a high-level protein expression from T7 promoter of pETIK. The pETIK has a lacI gene, which represses T7 RNA polymerase gene in the absence of IPTG. The regulation with lac repressor is beneficial to repress a basal level protein expression and to maintain a recombinant plasmid in BL21 (DE3) cell.

### Plasmid Map:



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### Features of T7 expression vectors

BioDynamics Laboratory Inc. provides 6 kinds of T7 expression vectors, pETUA, pETBA, pETIA, pETUK, pETBK, and pETIK. These vectors have the same multicloning site and specific feature of each vector is below:

	Plasmid copy number	Replicon	Antibiotic resistance	Feature and recommendation
pETUA	high copy	pUC	ampicillin	for non-toxic protein expression
pETBA	medium copy	pMB1	ampicillin	general expression
pETIA	medium copy	pMB1	ampicillin	stringent regulation with lac repressor
pETUK	high copy	pUC	kanamycin	for non-toxic protein expression
pETBK	medium copy	pMB1	kanamycin	general expression
pETIK	medium copy	pMB1	kanamycin	stringent regulation with lac repressor

### pETIK Sequence

GTTTGACAGC	TTATCATCGA	CTGCACGGTG	CACCAATGCT	TCTGGCGTCA	GGCAGCCATC	60
GGAAGCTGTG	GTATGGCTGT	GCAGGTCGTA	AATCACTGCA	TAATTCGTGT	CGCTCAAGGC	120
GCACTCCCCT	TCTGGATAAT	GTTTTTTGCG	CCGACATCAT	AACGGTTCCTG	GCAAATATTC	180
TGAAATGAGC	TGAGATCTCG	ATCCCGCGAA	ATTAATACGA	CTCACTATAG	GGAGACCACA	240
ACGGTTTCCC	TCTAGAAATA	ATTTTGTTTA	ACTTTAAGAA	GGAGATATA	ATATGCGGGG	300
TTCTCATCAT	CATCATCATC	ATGGTATGGC	TAGCATGACT	GGTGGACAGC	AAATGGGTCG	360
GGACGATGAC	GATAAGGATC	CCCGGGTACC	GAGCTCGAAT	TCGATTTTCGT	CGACAAGCTT	420
AGCGGCCGCC	GTTTAATCCG	GCTGCTAACA	AAGCCCAGAA	GGAAGCTGAG	TTGGCTGCTG	480
CCACCGCTGA	GCAATAACTA	GCATAACCCC	TTGGGGCCCTC	TAAACGGGTC	TTGAGGGGTT	540
TTTTGTCTGAA	AGGAGGAACT	ATATCCGGAT	GCGTTTCTAC	AAACTCTTTT	GTTTATTTTT	600
CTAAATACAT	TCAAATATGT	ATCCGCTCAT	GAGACAATAA	CCCTGATAAA	TGCTTATGTC	660
TGCTTACATA	AACAGTAATA	CAAGGGGTGT	TATGAGCCAT	ATTCAACGGG	AAACGTCTTG	720
CTCAAGGCCG	CGATTAAAT	CCAACATGGA	TGCTGATTTA	TATGGGTATA	AATGGGCTCG	780
CGATAATGTC	GGCAATCAG	GTGCGACAAT	CTATCGATTG	TATGGGAAGC	CCGATGCGCC	840
AGAGTTGTTT	CTGAAACATG	GCAAAGGTAG	CGTTGCCAAT	GATGTTACAG	ATGAGATGGT	900
CAGACTAAAC	TGGCTGACGG	AATTTATGCC	TCTTCCGACC	ATCAAGCATT	TTATCCGTAC	960
TCCTGATGAT	GCATGGTTAC	TCACCACTGC	GATCCCAGGG	AAAACAGCAT	TCCAGGTATT	1020
AGAAGAATAT	CCTGATTCAG	GTGAAAATAT	TGTTGATGCG	CTGGCAGTGT	TCCTGCGCCG	1080
GTTGCATTTCG	ATTCTGTGTT	GTAATTGTCC	TTTTAACAGC	GATCGCGTAT	TTCGTCTCGC	1140
TCAGGCCCAA	TCACGAATGA	ATAACGGTTT	GGTTGATGCG	AGTGATTTTG	ATGACGAGCG	1200
TAATGGCTGG	CCTGTTGAAC	AAGTCTGGAA	AGAAATGCAT	AAGCTATTGC	CATTCTCACC	1260
GGATTTCAGTC	GTCACATCATG	GTGATTTCTC	ACTTGATAAC	CTTATTTTTG	ACGAGGGGAA	1320
ATTAATAGGT	TGTATTGATG	TTGGACGAGT	CGGAATCGCA	GACCGATACC	AGGATCTTGC	1380
CATCCTATGG	AACTGCCTCG	GTGAGTTTTT	TCCTTCATTA	CAGAAACGGC	TTTTTCAAAA	1440
ATATGGTATT	GATAATCCTG	ATATGAATAA	ATTGCAGTTT	CATTTGATGC	TCGATGAGTT	1500
TTTCTAATTA	AAACATATAT	ACTTTAGATT	GATTTAAAAC	TTCATTTTTA	ATTTAAAAGG	1560

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ATCTAGGTGA	AGATCCTTTT	TGATAATCTC	ATGACCAAAA	TCCCTTAACG	TGAGTTTTTCG	1620
TTCCACTGAG	CGTCAGACCC	CGTAGAAAAG	ATCAAAGGAT	CTTCTTGAGA	TCCTTTTTTTT	1680
CTGCGCGTAA	TCTGCTGCTT	GCAAAACAAA	AAACCACCGC	TACCAGCGGT	GGTTTGTTTG	1740
CCGGATCAAG	AGCTACCAAC	TCTTTTTCCG	AAGGTAAC TG	GCTTCAGCAG	AGCGCAGATA	1800
CCAAATACTG	TCCTTCTAGT	GTAGCCGTAG	TTAGGCCACC	ACTTCAAGAA	CTCTGTAGCA	1860
CCGCCTACAT	ACCTCGCTCT	GCTAATCCTG	TTACCAGTGG	CTGCTGCCAG	TGGCGATAAG	1920
TCGTGTCTTA	CCGGGTGGA	CTCAAGACGA	TAGTTACCGG	ATAAGGCGCA	GCGGTCGGGC	1980
TGAACGGGGG	GTTCTGTCAC	ACAGCCCAGC	TTGGAGCGAA	CGACCTACAC	CGAACTGAGA	2040
TACCTACAGC	GTGAGCTATG	AGAAAGCGCC	ACGCTTCCCG	AAGGGAGAAA	GGCGGACAGG	2100
TATCCGGTAA	GCGGCAGGGT	CGGAACAGGA	GAGCGCACGA	GGGAGCTTCC	AGGGGAAAC	2160
GCCTGGTATC	TTTATAGTCC	TGTCGGGTTT	CGCCACCTCT	GACTTGAGCG	TCGATTTTTG	2220
TGATGCTCGT	CAGGGGGCG	GAGCCTATGG	AAAAACGCCA	GCAACGCGGC	CTTTTTACGG	2280
TTCTGTCCT	TTTGCTGGCC	TTTTGCTCAC	ATGTTCTTTC	CTGCGTTATC	CCCTGATTCT	2340
GTGGATAACC	GTATTACCGC	CTTTGAGTGA	GCTGATACCG	CTCGCCGCG	CCGAACGACC	2400
GAGCGCAGCG	AGTCAGTGAG	CGAGGAAGCG	GAAGAGCGCC	TGATGCGGTA	TTTTCTCCTT	2460
ACGCATCTGT	GCGGTATTTT	ACACCGCATA	TATGGTGCAC	TCTCAGTACA	ATCTGCCTCTG	2520
ATGCCGCATA	GTTAAGCCAG	TATACACTCC	GCTATCGCTA	CGTGACTGGG	TCATGGCTGC	2580
GCCCCGACAC	CCGCCAACAC	CCGCTGACGC	GCCCTGACGG	GCTTGTCTGC	TCCCCGCATC	2640
CGCTTACAGA	CAAGCTGTGA	CCGTCTCCGG	GAGCTGCATG	TGTCAGAGGT	TTTACCCTGC	2700
ATCACCGAAA	CGCGCGAGGC	AGCAGATCAA	TTCGCGCGCG	AAGGCGAAGC	GGCATGCATT	2760
TACGTTGACA	CCATCGAATG	GTGCAAAACC	TTTCGCGGTA	TGGCATGATA	GCGCCCGGAA	2820
GAGAGTCAAT	TCAGGGTGGT	GAATGTGAAA	CCAGTAACGT	TATACGATGT	CGCAGAGTAT	2880
GCCGGTGTCT	CTTATCAGAC	CGTTTCCCGC	GTGGTGAACC	AGGCCAGCCA	CGTTTCTGCG	2940
AAAACGCGGG	AAAAAGTGGA	AGCGGCGATG	GCGGAGCTGA	ATTACATTCC	CAACCGCGTG	3000
GCACAACAAC	TGGCGGGCAA	ACAGTCGTTG	CTGATTGGCG	TTGCCACCTC	CAGTCTGGCC	3060
CTGCACGCGC	CGTCGCAAAT	TGTCGCGGCG	ATTAAATCTC	GCGCCGATCA	ACTGGGTGCC	3120
AGCGTGGTGG	TGTCGATGGT	AGAACGAAGC	GCGCTCGAAG	CCTGTAAAGC	GGCGGTGCAC	3180
AATCTTCTCG	CGCAACGCGT	CAGTGGGCTG	ATCATTAACT	ATCCGCTGGA	TGACCAGGAT	3240
GCCATTGCTG	TGGAAGCTGC	CTGCACTAAT	GTTCCGGCGT	TATTTCTTGA	TGTCTCTGAC	3300
CAGACACCCA	TCAACAGTAT	TATTTTCTCC	CATGAAGACG	GTACGCGACT	GGGCGTGGAG	3360
CATCTGGTTC	CATTGGGTCA	CCAGCAAATC	GCGCTGTTAG	CGGGCCCAT	AAGTTCTGTC	3420
TCGGCGCGTC	TGCGTCTGGC	TGGCTGGCAT	AAATATCTCA	CTCGCAATCA	AATTCAGCCG	3480
ATAGCGGAAC	GGGAAGGCGA	CTGGAGTGCC	ATGTCGGGTT	TTCAACAAAC	CATGCAAATG	3540
CTGAATGAGG	GCATCGTTCC	CACTGCGATG	CTGGTTGCCA	ACGATCAGAT	GGCGCTGGGC	3600
GCAATGCGCG	CCATTACCGA	GTCCGGGCTG	CGCGTTGGTG	CGGATATCTC	GGTAGTGGGA	3660
TACGACGATA	CCGAAGACAG	CTCATGTTAT	ATCCCGCCGT	CAACCACCAT	CAAACAGGAT	3720
TTTCGCCTGC	TGGGGCAAAC	CAGCGTGGAC	CGCTTGCTGC	AACTCTCTCA	GGGCCAGGCG	3780
GTGAAGGGCA	ATCAGCTGTT	GCCCGTCTCA	CTGGTGAAAA	GAAAAACCAC	CCTGGCGCCC	3840
AATACGCAAA	CCGCCTCTCC	CCGCGCGTTG	GCCGATTTCAT	TAATGCAGCT	GGCAGCAGAG	3900
GTTTCCCGAC	TGGAAAAGCGG	GCAGTGAGCG	CAACGCAATT	AATGTGAGTT	AGCGCGAATT	3960
GATCTG						3966

## PRODUCT INFORMATION

### PRODUCT USAGE

#### Cloning of a gene to pETIK:

Below is the multiple cloning site of pETIK. To express a recombinant protein correctly, it is necessary to clone the gene of interest in frame with an N-terminal peptide of pETIK. The start codon of pETIK is boxed ATG in the below figure. Digest pETIK completely with appropriate restriction enzyme(s) to form DNA ends which can be ligated to the gene of interest. If only one restriction enzyme is used, dephosphorylation of a vector is often performed. Ligation of processed pETIK and the gene of interest can be performed by the standard procedure. The following transformation procedure should be done with non-expression hosts such as DH5 $\alpha$  or JM109. In the transformation, recombinant cells should be selected on LB agar plates containing 15-25  $\mu$ g/ml of kanamycin, because higher concentration of kanamycin often retarded cell growth on the agar plates. Recombinant plasmids derived from pETIK are selected by colony-PCR, enzyme digestion of prepared plasmids, or other methods. Sequencing of cloning portion and an insert region on the obtained plasmid is recommended to determine the correct recombinant plasmids for expression experiments.

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                T7 promoter                                XbaI
GATCCCGCGA AATTAATACG ACTCACTATA GGGAGACCAC AACGGTTTCC CTCTAGAAAT 259
AspProAlaL ysLeuIleAr gLeuThrIle GlyArgProG lnArgPhePr oSerArgAsn
                NdeI                                6xHis
AATTTTGTTT AACTTTAAGA AGGAGATATA CATATGCGGG GTTCTCATCA TCATCATCAT 319
AsnPheVal* **Leu***Gl uGlyAspIle HisMetArgG lySerHisHi sHisHisHis
                EK                                BamHI
CATGGTATGG CTAGCATGAC TGGTGGACAG CAAATGGGTC GGGACGATGA CGATAAGGAT 379
HisGlyMetA laSerMetTh rGlyGlyGln GlnMetGlyA rgAspAspAs pAspLysAsp
                KpnI                                EcoRI                                SalI                                NotI                                ↑
CCCCGGGTAC CGAGCTCGAA TTCGATTTTCG TCGACAAGCT TAGCGGCCGC CGTTTAAATCC 439
                SmaI                                SacI                                HindIII
ProArgValP roSerSerAs nSerIleSer SerThrSerL euAlaAlaAl aVal***Ser

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EK: Enterokinase recognition sequence (AspAspAspAspLys↓)

ATG: start codon

TAA: stop codon

#### Protein Expression Procedure :

The following protocol is a general guide for the protein expression by using T7 expression vectors, pETUA, pETBA, pETIA, pETUK, pETBK, and pETIK, coupling with an expression host *E. coli* cell, BL21(DE3) cells or BL21(DE3)pLysS cells.

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- Before starting:

Transform BL21(DE3) or BL21(DE3)pLysS cells with the prepared expression plasmid by the standard procedure. If kanamycin is used for selection, recombinant cells should be selected on LB agar plates containing 15-25 µg/ml of kanamycin, because a higher concentration of kanamycin often retards cell growth on the agar plates.

‡ Notes for transformation

1. Sometimes, expression may vary among transformants. If large and small colonies are observed in the same plate, the expressed protein may affect the growth of the *E. coli* cells.

2. If the expressed protein is toxic to *E. coli* cells, transformants may not be obtained.

In this case, repression of a basal level expression by T7 promoter may work, see “Notes for expression.”

- Expression:

1. Following transformation, pick a colony and inoculate it into 3 ml of LB medium containing the appropriate antibiotic (kanamycin is often used at 25-30 µg/ml for liquid culture) with shaking at 37°C, overnight. For the BL21(DE3)pLysS strain, it is preferable to add chloramphenicol at a final concentration of 34 µg/ml in the overnight culture to maintain pLysS.

2. The next morning, transfer 0.5 ml of the overnight culture to a new 10 ml of LB medium containing the appropriate antibiotic to select the expression plasmid. Grow the culture with shaking at 37°C until the OD<sub>600</sub> reaches 0.5 (approximately 2 hrs but this depended on the expression plasmids).

When using BL21(DE3)pLys, chloramphenicol is not usually required in the short-period culture.

3. When the OD<sub>600</sub> reaches 0.5, transfer an aliquot (e.g., 1 ml) of the culture to a new centrifuge tube and centrifuge it to harvest cells. Store the cells at -80°C until analysis.

Add IPTG to a final concentration of 1 mM to the rest of the culture and grow the culture with shaking at 37°C for 3 hours.

The IPTG concentration and induction time are general values. It is recommended to determine the optimal condition for the target gene expression.

4. After the induction, harvest the cells. To analyze the expression, before harvesting the cells, transfer an aliquot of the culture (e.g., 1 ml) and centrifuge it to precipitate the cells.

- Analysis

1. Suspend the precipitated cells (from the 1 ml culture) in 200 µl of 1× PBS buffer.

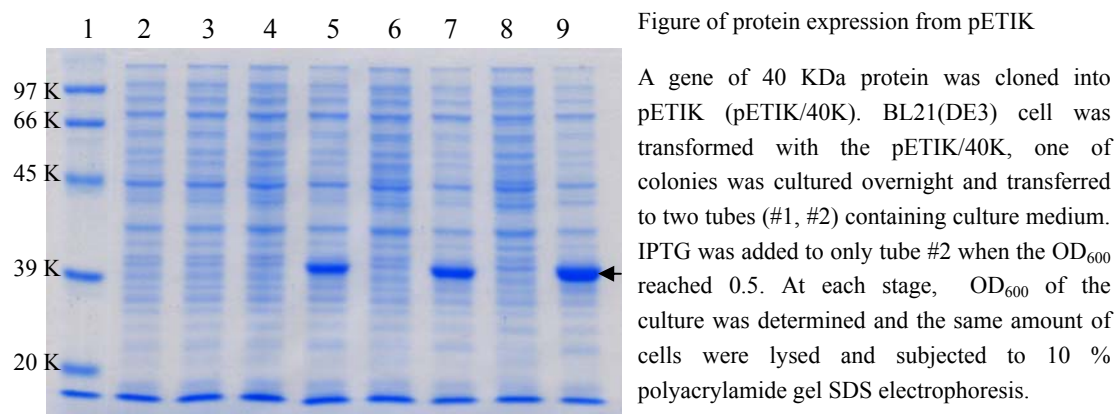
2. Mix an aliquot of the suspension (e.g., 100 µl) with an equal volume of 2 × SDS sample buffer.

3. Heat the mixture at 85°C for 5 min, then centrifuge at 10,000 g for 10 min. Subject the supernatant (e.g., 5-25 µl) to SDS-PAGE. Western blot will help analyzing the expression of the target protein.

- 2 × SDS sample buffer : 2 % sodium dodecyl sulfate, 5 % 2-mercaptoethanol, 20 % glycerol, 0.02 % BPB, 62.5 mM Tris-HCl, pH6.8

- 1× PBS buffer.: 20 mM sodium phosphate, 150 mM sodium chloride, pH7.4

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An arrow shows the expressed 40 KDa protein. Only induced cells expressed 40 KDa proteins.

Lane 1: DynaMarker Protein Eco (#DM610)  
Lane 2, 3: Cells from tubes #1 and 2 before induction.  
Lane 4: Cells (tube #1), 1 hour after OD<sub>600</sub> reached 0.5  
Lane 5: Cells (tube #2), 1 hour after induction  
Lane 6: Cells (tube #1), 2 hours after OD<sub>600</sub> reached 0.5  
Lane 7: Cells (tube #2), 2 hours after induction  
Lane 8: Cells (tube #1), 4 hours after OD<sub>600</sub> reached 0.5  
Lane 9: Cells (tube #2), 4 hours after induction

### ‡ Notes for expression:

1. As the T7 expression method is a high-level protein expression system, some basal level expression of the target protein will occur in uninduced cells. This is likely problematic in cases in which the target protein is toxic to *E. coli* cells. In this case, it may be necessary to decrease the basal level expression as follows:

- Use a lower-copy number T7 expression vector, pETBA, pETBK, but not pETUA, pETUK
- Use a stringent regulated expression vector, pETIA, pETIK.
- Use liquid medium and agar plates supplemented with glucose (0.5 -1 %).  
Glucose is known to decrease a basal expression from *lacUV5* promoter<sup>2)</sup>.
- Use BL21(DE3)pLysS strain but not BL21(DE3) strain.

The T7 Lysozyme encoded in a pLysS plasmid reduces the basal level of T7 RNA polymerase Expression<sup>3)</sup>. This leads to suppression the basal level expression of the target protein.

2. When expressing proteins in BL21(DE3) cells, if it takes a longer time (5 hrs or more) to reach 0.5 at OD<sub>600</sub> after inoculating the overnight culture (0.5 ml) to a new LB medium (10 ml), the expressed protein is likely toxic to *E. coli* cells.

3. When BL21(DE3) cells lyse after induction with IPTG, the expressed protein is likely toxic to *E. coli* cells.

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### Reference:

- 1) Studier, F.W. and Moffatt, B.A., *J. Mol. Biol.* 189 (1986) 113–130.
- 2) Pan, S. and Malcom, B.A., *BioTechniques* 29 (2000), 1234–1238
- 3) Moffatt, B.A. and Studier, F.W., *Cell* 49 (1987) 221-227

General reference in this Product Information

Sambrook, J. and Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

### Related Products:

DV210	Expression Vector pETBA	DV215	Expression Vector pETIA
DV220	Expression Vector pETUK	DV230	Expression Vector pETBK
DV235	Expression Vector pETIK	DS110	DNA Ligation Kit ver. 2
DS210	Competent Cell JM109	DS220	Competent Cell DH5 $\alpha$
DS225	Jet Competent Cell (DH5 $\alpha$ )	DS240	Competent Cell BL21
DS255	Zip Competent Cell BL21(DE3)	DS260	Competent Cell BL21(DE3)pLysS
DS500	QuickBlue Protein Staining Solution		

### ● Purchaser Notification

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