Expression and Purification of subtilisin-like protease

Gene and Expression Vector

1.1 Gene coding for the target protein

The cDNA coding for the target protein was chemically-synthesized with optimization for expression in *E.coli* and described as below.

> 12_subtilisin-like_protease-1172bp

atgagcgataaaattattcacctgactgacgacagttttgacacggatgtactcaaagcggacgggg
cgatcctcgtcgatttctgggcagagtggtgcggtccgtgcaaaatgatcgccccgattctggatgaaatcg
ctgacgaatatcagggcaaactgaccgttgcaaaactgaacatcgatcaaaaccctggcactgcgccgaaa
tatggcatccgtggtatcccgactctgctgctgttcaaaaacggtgaagtggcggcaaccaaagtgggtgc
actgtctaaaggtcagttgaaagagttcctcgacgctaacctggccggttctggttctggccatatgcaccat
catcatcatcattcttctggtctggtgccacgcggttctggtatgaaagaaaccgctgctgctaaattcgaacg
ccagcacatggacagcccagatctgggtaccgacgacgacgacaaggCCATGGCCACCGCGCCG
ATGCTGCGTCTGGATAGCAAGACCGCGATCCCGAACGAGTACATTGTGGTTCTGCAG
AAAAACCTGACCAACTATGCGGTGGGCAAGCACATGAACAGCGTTAAAACCCTGCT
GACCGGCACCAACGACACCAAGATCCTGTTCGAGCACAACTTCCGTTGGTTTAAGG
CGTACAGCATCCGTACCAACCAGAAAATGATTCGTCACCTGGCGGAGCAACCGGAA
GTGCGTTACATTGAAGCGAACCAGGTGGTTAAAGCGTATCAGGCGCAATGCCAGGA
TCAACTGGAGGCGACCTGGGGTCTGGTGCGTACCGTTGAACGTGACCTGCTGCTGG

ATGGCATCTACCACTATCAAGGTGGCGACGGTGAAGGCGTTGATGCGTACATCATTG
ACACCGGTATTTATACCGAGCACAGCGAATTTGGTGGCCGTGCGAAGTGGGGCGTG
GACACCGTTGATACCCCGAGCCCGGAGACCGATGAAAACGGTCACGGCACCCATGT
GGCGGGTACCGTTATGAGCGATGCGTGGGGCCTGGCGAAGAAAGCGACCGCGATC
GCGGTGAAAGTTCTGAGCCGTAGCGGTAGCGGTAGCACCGCGGGTGTGATTGAGG
GCGTTGAATGGACCGGTCAGCAACACAGCGGCAACAACAAGAAAAGCGTGGCGA
ACATGAGCCTGGGTGGCGGTCGTAGCGATGCGATGAACGAGGCGGTTAAGGCGGT
GGTTGAAGCGGGTGTGGTTATGGTGGTTGCGGCGGGTAACGAAGGTTGGGAAGCG
TGCAACGTGAGCCCGGCGAGCGAGCCGACCGCGATCACCGTTGGTTGCAGCGATA
ACGAAGACGATTTCTGCTTCTTTAGCAACTATGGTACCTGCATGGATATCATTGCGCC
GGGTCAGGATGTGACCAGCGCGTGGATCGGCGGTCAATTTGCGGACAACACCATTA
GCGGTACCAGCATGAGCGCGCCGCACATTGCGGGTATTGTTGCGAAGTACATGAGC
CGTCAGAGCAGCGTGCCGAGCCCGGAGGAAGTTAAAGATTTCCTGCAGACCACCA
GCACCAAGGACAAAATCAACCAAATTCCGAGCAACAGCGACACCCTGAACTATCTG
GGTTTTATGGATTGCGGCGGTGGCCCGGACGTGTAACTCGAG

1.2 Expression vector

The cDNA sequence described in the §1.1 was cloned in pET32a expression vector.

> 12_subtilisin-like_protease(547AAs,58.84kDa, pI:5.13)

MSDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTV

AKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLAGSGSGH

MHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTDDDDKAMATAPMLRLD

SKTAIPNEYIVVLQKNLTNYAVGKHMNSVKTLLTGTNDTKILFEHNFRWFKAYSIRTNQ

KMIRHLAEQPEVRYIEANQVVKAYQAQCQDQLEATWGLVRTVERDLLLDGIYHYQGG

DGEGVDAYIIDTGIYTEHSEFGGRAKWGVDTVDTPSPETDENGHGTHVAGTVMSDA

WGLAKKATAIAVKVLSRSGSGSTAGVIEGVEWTGQQHSGNNKKSVANMSLGGGRSD

AMNEAVKAVVEAGVVMVVAAGNEGWEACNVSPASEPTAITVGCSDNEDDFCFFSNY

GTCMDIIAPGQDVTSAWIGGQFADNTISGTSMSAPHIAGIVAKYMSRQSSVPSPEEVK

DFLQTTSTKDKINQIPSNSDTLNYLGFMDCGGGPDV

Features: Trx-His-EK:[1:159]

Expression Tests

2.1 Aim

-Determination of optimal conditions for protein expression by evaluation of:

*Induction strategy

*Temperature and time for induction

**E.coli* strain used for protein production

-Identification of best extraction condition: native or denatured

2.2 Short protocol description

-Culture:

*Bacteria starter obtained by incubation at 37°C

*IPTG induction with different temperatures and incubation times (see details in §2.3).

-Protein samples preparation (for each expression condition tested):

*Bacteria harvested by centrifugation

*Lysis with native buffer

*Clarification (centrifugation C1): The supernatant is the native protein extract (NPE)

*Solubilization of the pellet from C1 with a denaturing buffer (Urea 8M)

*Clarification (centrifugation): The supernatant is the denatured protein extract (DPE)

*PAGE analysis of NPE and DPE.

2.3 Results of the expression tests

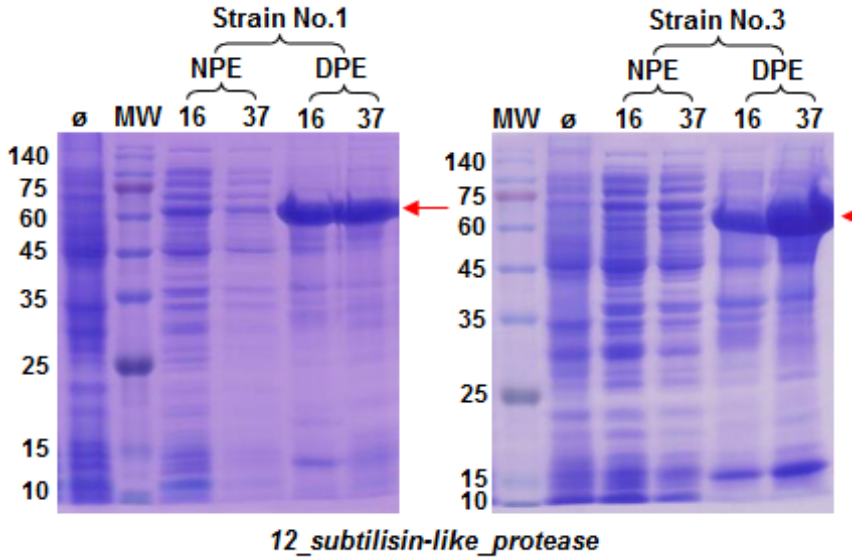

Figure 1. Expression tests of the target protein. Analysis of NPE and DPE prepared as described in §2.2.

MW. Molecular weight marker. **Ø.** Non-induced bacteria culture (negative control).

16 and 37. Incubation temperature (°C) during induction with IPTG.

Induction with IPTG 1mM during 16h at 16°C, or during 4h for other temperatures.

2.4 Conclusion of the expression tests

Note: *E.coli strains tested are intended to overcome one or several expression issues such as (but not limited to) expression level, solubility or toxicity.*

All the protein had expressed, most were in denatured condition. Optimal

expression condition was described as below.

	Strain No.	Temperature	Induction Time
12_subtilisin-like_protease (DPE)	No.3	37°C	4h

Table1. Optimal denatured expression conditions of target protein.

Small-scale purification tests

3.1 Aim

200ml purification tests in DPE conditions for target protein. Starting material: DPE prepared after production in optimal denatured expression conditions described in the Table 1.

3.2 Short protocol description

-Lysis buffer: PBS, pH7.5, 10% Glycerol

-Washing buffer: PBS, pH7.5., 1% Triton X 100, 5 mM EDTA

-Denaturing buffer: PBS, pH7.5, 8M urea(or 2% Sarcosyl)

-Dialysis buffer: PBS, pH7.5, 0.02% Sarcosyl

-Analysis by SDS-PAGE of fractions of interest

-Final sample QC: qualitative by SDS-PAGE, quantitative by Bradford method.

3.3 Results of the purification tests

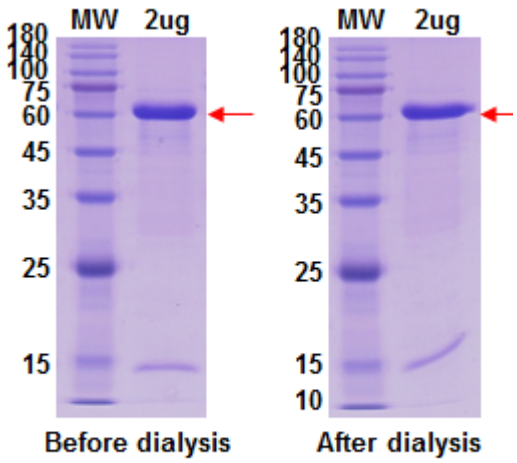

Figure 3. Final sample QC of denatured condition. *Coomassie blue staining.*

Reducing-PAGE analysis. 2μg of sample loaded.

3.4 Conclusion of the purification tests

Final sample details (200ml production)

For 12_subtilisin-like_protease protein,

-Final production: 1.7mg/ml, 2mg/vial, 1vial ;

-Total: 2mg

-Final buffer: 0.02% Sarcosyl