Expression and Purification of endo-beta-1,4-glucanase

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.

> 14_endo-beta-1_4-glucanase-2178 bp

atgtcccctatactaggttattggaaaattaagggccttgtgcaacccactcgacttcttttggaatatctt
gaagaaaaatatgaagagcatttgtatgagcgcgatgaaggtgataaatggcgaaacaaaaagtttgaatt
gggtttggagtttcccaatcttccttattatattgatggtgatgttaaattaacacagtctatggccatcatacgtt
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gcggttttggatattagatacggtgtttcgagaattgcatatagtaaagactttgaaactctcaaagttgatttt
cttagcaagctacctgaaatgctgaaaatgttcgaagatcgtttatgtcataaaacatatttaaatggtgatcat
gtaacccatcctgacttcatgttgtatgacgctcttgatgttgttttatacatggacccaatgtgcctggatgcgt
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atatagcatggcctttgcagggctggcaagccacgtttggtggtggcgaccatcctccaaaatcggatctgg
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CAGAACTGCTTTGGTAGCCCGGTGAGCAGCCCAATCAGCCCAATGGCCGGTCGTGA
AAGCCTGGGTACCAGCCCGGTGAGCAGCCCGAGCAAGGACAAATACGGCGACCGT
TTCATTCCGAGCCGCGCTGGTGCTAACTGGGAGATCGGTTTTAACAGCATTCAGGGC

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ACGGCAAGGATGGTCTGGCCTACACCTGCCTGCTGAAAAACGAGCTGCTGGGCGCG

GGTATCGAAGACCTGAAGGAGCAGACCGAAGATCGTCGCGGTGTGCTGAGCCCAAC

CACCCCGGAAAAGCGTAACCTGTTCCGCTACCACCTGACCGCTAAACAGGCCAGCC

CGGAGAACACCGACCACCTGAGCCCGTATAGCCTGAGCCCGGTGGGCAAGAAAAG

CCAGAAGCTGCTGCGTAGCCCGCGCAAACAGACCCGTAAGATCAGCAAAATTCCGT

TCAAAGTGCTGGATGCCCCAGAGCTGCAGGATGACTTCTACCTGAACCTGGTGGATT

GGAGCGCCACCAACATCCTGAGCGTGGGCCTGGGTACCTGCGTGTATCTGTGGAGCG

CGTGCACCAGCCAGGTGACCCGCCTGTGCGACCTGAGCTGCGATGGCGACAGCGTG

ACCAGCGTGAACTGGAACGAACGTGGTAACCTGGTGGCGGTGGGTACCCACAAGG

GTTACGTGCAAGTGTGGGACGCGATGGCTGGTAAACGTATTAGCATGCTGGAGGGTC

ACAGCGCGCGCGTGGGCGCCCTGGCGTGGAACGCTGATATCCTGAGCAGCGGCAGC

CGTGACCGCCTGATTCTGCAGCGCGATGTGCGCACCCCGAGCGTGGTGCCAGAGCG

TCGCCTGGCTGGTCACCGTCAGGAAGTGTGCGGCCTGAAGTGGAGCCCGGACCACC

AGCACCTGGCTAGCGGCGGTAACGATAACAAACGCATCGTGTATAAGAAAGGTACCT

TCGTGGACAGCTTTCGTCCGACCAACATGCGCTGCACCGCTCTGATTCTGCTGGCCCT

GCTGGGTGCGGCGCGTGCCCAGAGCTACTATGAAGTGACCGGCGACTGGGGCGACC

GTTTCCAGGCGAACGTGTACATCCCGGTGAGCCAGGCGGTGAATGGTTGGACCGCG

ACCCTGAAGTTTGATAAAGCTGTGGAGCTGGAAATTTGGCTGGCCGAAGTGACCAG

CACCAGCGACGGCAACACCGTGTTCGAGCTGAAGGATATGGGTTGGAATGCTAACG

TGCCAGCCGGCACCTTCGAACTGAGCTTTATCGCCAACACCCAGGGCAGCGCCGCT

AACCTGGTGGGTCTGTGGTTTAATGGTCAGGAAGTGACCAGCGGCGGCACCGGCAC

CGGCACCGGTACCGGCACTGGCACTGGTACCACCCATCGTCCAAGCACCGTGAGCA

CCAGCCATACCCATGCTACCGCTCCAACCGCCGCTCAGAGCGGTACCACCCTGGGTG

TGGTGCCGCCGGTGGTGGGCGCCTACGACTATGTGGATGCGCTGGCTAAGAGCATTC

TGTTCTATGAGGCCCAGCGTGCTGGTAACCTGCCGGGTAACAATCGTGTGCCATGGC

GTCGCAGCTGCTGCCAGGGCGACGGTCAGGATGTGGGTCTGGACCTGAGCGGCGGT

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GTGCTGAGCTGGGGTATGATTGAGTTCAAGCGTGCTTATGAAGCGGCCGGTGAAATG

GCTAATGCCCTGGACAGCATCAAATGGGTGACCGATTACCTGATTAAGTGCCACCCG

AGCAAATTCGAGTTTGTGGCCCAGGTGAGCACCCACGGTTACAGCCAGAACCAGAT

CCTGGTGTGGAAGTATCCGAGCCTGGTGCAGGTGGCTAAACTGACCGGTCACAGCTA

CCGTGTGCTGTATCTGGCCATGAGCCCAGATGGTGAAGCCATTGTGACCGGTGCTGG

CGACGAAACCCTGCGTTTCTGGAACGTGTTTAGCAAGACCCGCAGCAACAAGGAGA

GCAAAAGCGTGCTGAACCTGTACACCCACATCCGTTAACTCGAG

1.2 Expression vector

The cDNA sequence described in the §1.1 was cloned in pGEX-6P-1 expression vector.

>14_endo-beta-1_4-glucanase (947AAs,105.4kDa, pI:8.16)

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPY

YIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETL

KVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFP
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GFNSIQGMYEKTSGQARKAREANSDNGKDGLAYTCLLKNELLGAGIEDLKEQTEDRRG
VLSPTTPEKRNLFRYHLTAKQASPENTDHLSPYSLSPVGKKSQKLLRSPRKQTRKISKIPF
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VNWNERGNLVAVGTHKGYVQVWDAMAGKRISMLEGHSARVGALAWNADILSSGSR
DRLILQRDVRTPSVVPERRLAGHRQEVCGLKWSPDHQHLASGGNDNKRIVYKKGTFV
DSFRPTNMRCTALILLALLGAARAQSYYEVTGDWGDRFQANVYIPVSQAVNGWTATL
KFDKAVELEIWLAEVTSTSDGNTVFELKDMGWNANVPAGTFELSFIANTQGSAANLVG
LWFNGQEVTSGGTGTGTGTGTGTTHRPSTVSTSHTHATAPTAAQSGTTLGVVPPVV
GAYDYVDALAKSILFYEAQRAGNLPGNNRVPWRRSCCQGDGQDVGLDLSGGWFDA
GDHLKLHFPLSYTITVLSWGMIEFKRAYEAAGEMANALDSIKWVTDYLIKCHPSKFEFV
AQVSTHGYSQNQILVWKYPSLVQVAKLTGHSYRVLYLAMSPDGEAIVTGAGDETLRFW
NVFSKTRSNKESKSVLNLYTHIR

Features：GST tag:[1:218]

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

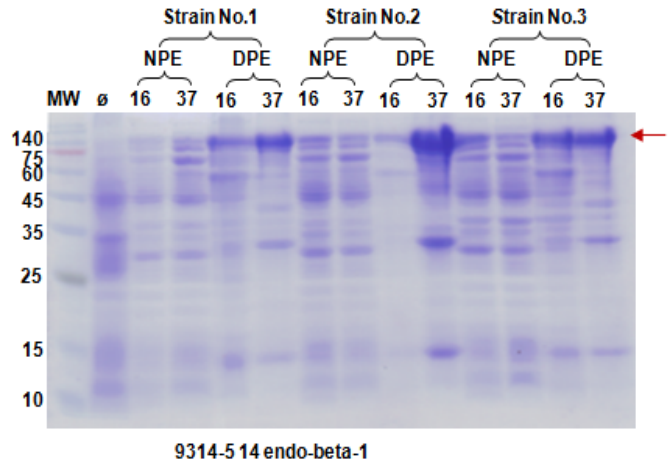

Figure 1. Expression tests of the target protein. Analysis of NPE and DPE prepared as described in §2.2.

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

+. Western blot internal control (Unrelated His-Tagged protein).

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.

Optimal expression condition was described as below.

	Strain No.	Temperature	Induction Time
14 endo-beta-1(NPE&DPE)	No.2	37°C	4h

Table1. Optimal native expression conditions of target protein.

Small-scale purification tests

3.1 Aim

200mL purification tests in NPE&DPE conditions for target protein. Starting material: NPE&DPE prepared after production in optimal native expression conditions described in the Table 1.

3.2 Short protocol description

For native purification,

Purification by affinity vs. GST-Tag on GST resin

-Wash: 50 mM Tris-HCl pH 8.0, 150 mM NaCl

-Elution: 50 mM Tris-HCl, 150 mM NaCl, 15 mM reduced Glutathione

-After purification, the samples of interest are buffer exchanged vs. TBS pH8.0

For denatured purification,

-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%NLS)

-Dialysis buffer: PBS, pH7.5, 8M urea (or 0.2%NLS)

-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

For 14 endo-beta-1 (Native and Denatured),

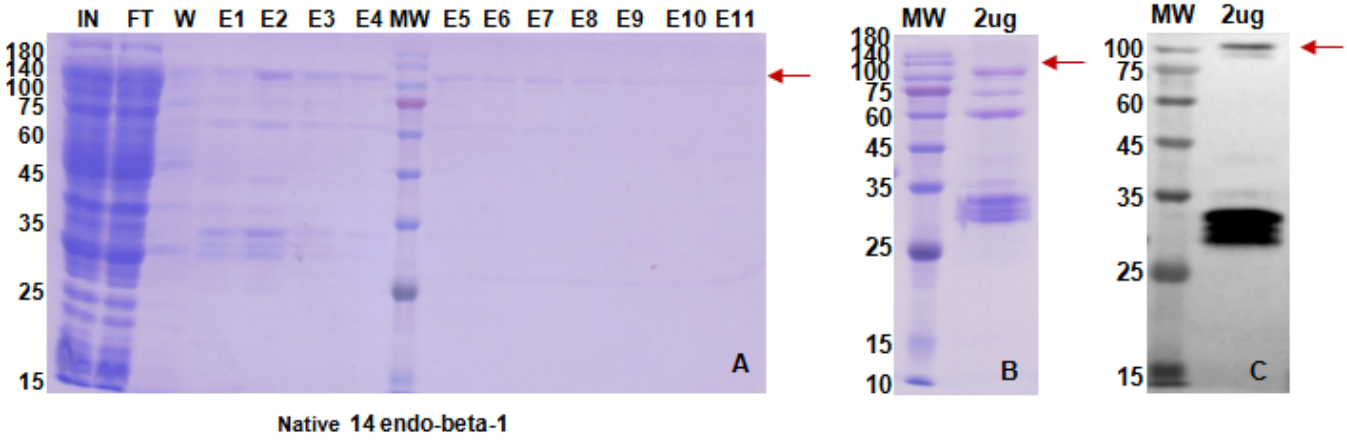

Figure 2. Purification tests of target protein. SDS-PAGE with Coomassie blue staining.

MW. Molecular weight marker. **IN.** Input. **FT.** Flow through. **W1-W3.** Wash fractions. **E1-E9.** Eluted fractions.

A. Purification profiles (Reduced analysis). **B and C.** Final samples QC after buffer exchange (2µg per lane).

A and B. Coomassie blue staining. **C.** Western blot with anti GST-Tag antibody (ECL revelation).

Target protein was obtained from native condition, but the yield and purity were low.

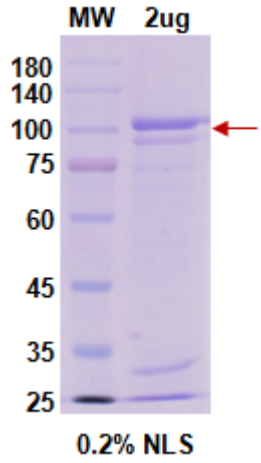

Figure 8. Final sample QC of denatured condition. Coomassie blue staining. Reducing-PAGE and WB analysis. 2μg of sample loaded.