Expression and Purification of cathepsin B

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.

> 2_cathepsin_B-986bp

CCATGGCCAAGGAGTTCCCGATCCACCAGCCGCTGACCCAAGAAATCATTGATT
ACGTGAACACCATTGACACCACCTGGAAGGCGGGTTGGAACTTTCAGGGCGCGAC
CGTGAGCTATGTTAAAGGTCTGTGCGGCGTTATCCGTGACCCGAACAACCACAAGCT
GCCGCTGAAACTGCACGAGCTGAACGCGCAGGACATCCCGGATACCTTCGACAGCC
GTACCCAATGGGCGAACTGCCCGACCATTAAAGAGGTGCGCGATCAAGGTAGCTGC
GGTAGCTGCTGGGCGCTGGCGGCGGTGGAAGCGATGAGCGACCGTATCTGCGTTGC
GAGCAAGGGTAGCACGATGGCGCACATTAGCGCGGAAGATCTGAACAGCTGCTGC
AAAAGCTGCGGTAACGGTTGCAACGGTGGCTTCCCGGAGGCGGCGTGGGAATACT
GGAAACGTGATGGTCTGGTTACCGGTGGCCCGTATGGTAGCCACCAGGGCTGCCAA
CCGTATGAGATCAAGCCGTGCGAACACCACATTAACGGTAGCCGTCCGGCGTGCGGT
AAACTGGAGCCGACCCCGCGTTGCAAGAAAAGCTGCGAAAGCGGTTATAACGTTAC
CTTTGCGAAGGACAAACACTACGCGAAGACCGCGTATAGCGTGAGCAGCAAAGTT
CAGCAAATCCAGATGGAGATTATGACCAACGGTCCGGTGGAAGCGGCGTTCACCGT
TTACGCGGATTTTCCGCACTACAAGAGCGGTGTGTATCAACATGAGAGCGGTGCGG

AACTGGGTGGCCACGCGGTTAAAATGATCGGTTGGGGCACCGAGGGTAGCACCCC
GTATTGGCTGATTGCGAACAGCTGGAACACCGACTGGGGTAACATGGGCTTCTTTA
AGATCCTGCGTGGTCAGGATGAGTGCGGCATCGAACGTGACATTGTTGCGGGTGAA
CCGAAACTGGATCTCGAGcaccaccaccaccaccactga

1.2 Expression vector

The cDNA sequence described in the §1.1 was cloned in pET28b expression vector.

> 2_cathepsin_B(327AAs,36.2kDa,pI:6.17)

MAKEFPIHQPLTQEIIDYVNTIDTTWKAGWNFQGATVSYVKGLCGVIRDPNNHK
LPLKLHELNAQDIPDTFDSRTQWANCPTIKEVRDQGSCGSCWALAAVEAMSDRICVA
SKGSTMAHISAEDLNSCCKSCGNGCNGGFPEAAWEYWKRDGLVTGGPYGSHQGCQ
PYEIKPCEHHINGSRPACGKLEPTPRCKKSCESGYNVTFAKDKHYAKTAYSVSSKVQQI
QMEIMTNGPVEAAFTVYADFPHYKSGVYQHESGAELGGHAVKMIGWGTEGSTPYWL
IANSWNTDWGNMGFFKILRGQDECGIERDIVAGEPKLDLEHHHHHH

Features: His-tag with linker:[320:327]

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

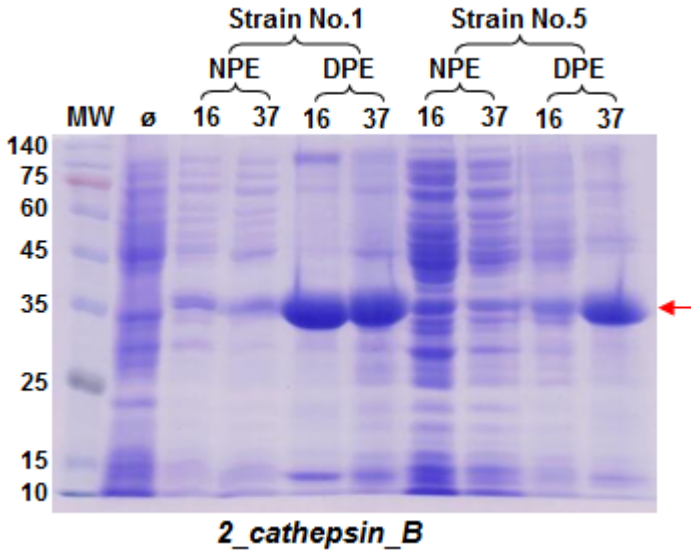

Figure 1. Expression tests of the target protein. Analysis of NPE and DPE prepared as described in §2.2.

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.*

The protein had expressed,optimal expression condition was described as below.

	Strain No.	Temperature	Induction Time
2 cathepsin_B (DPE)	No.1	37°C	4h

Table1. Optimal denatured expression conditions of target protein.

Small-scale purification tests

3.1 Aim

1L 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

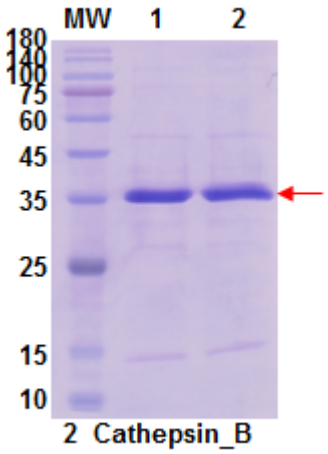

Figure 3. Final sample QC of denatured condition. *Coomassie blue staining.*

Reducing-PAGE analysis. 2μg of sample loaded.1. Before dialysis 2. After dialysis