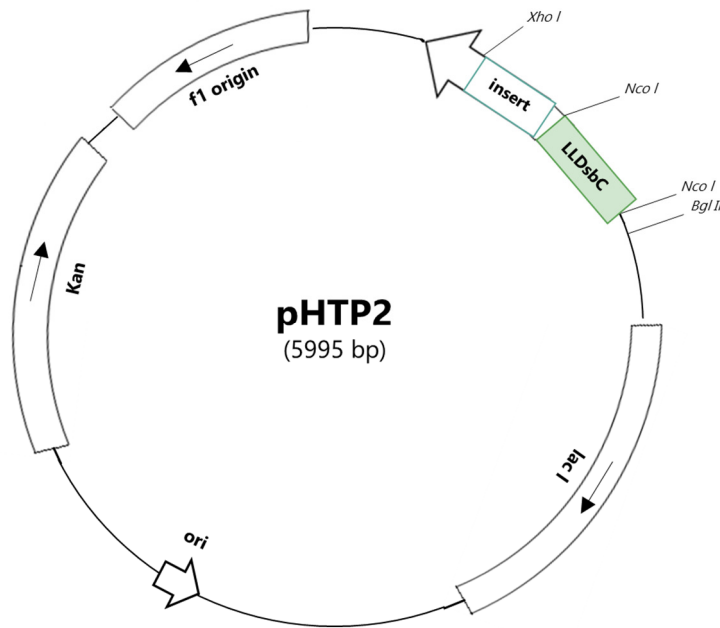


pHTP2 Expression Vector

pHTP2 was designed for the cloning and expression of high-levels of recombinant proteins in *Escherichia coli*. Recombinant proteins are expressed in fusion with the leader less disulfide-bond isomerase (LLDsbC), which is able to promote solubility and folding of disulfide bond-containing partners in the cytoplasm. This vector, included in the portfolio of NZYTech pHTP expression vectors, is part of the NZYEasy Cloning & Expression System. pHTP2 contains two poly-histidine (6xHis) sequences (N- and C-terminal) which allow subsequent recombinant protein purification by immobilized metal ion affinity chromatography (IMAC).

1. Vector Map



pHTP2 Cloning/Expression Region

<i>Nco I</i>	LLDsbC	<i>Nco I</i>	His-Tag
<u>CCATGGG</u> GAGATGACGCGGCAATTCACAAACG. 648bp. AAAATGACCAGCGGTAAAGGATCAT		<u>CCATGGG</u> CAGCAGCCATCATCATCATCACAGCAGCGGC	
MetGlyAspAspAlaAlaIleGlnGlnThr. 216aa. LysMetThrSerGlyLysGlySerSerMetGlySerSer		HisHisHisHisHisHisHisSerSerGly	
CCTCAGCAAGGGCTGAGG / ⚡ / CCTCAGCTTCCGCTGAGGTCGTCGACAAGCTTGCGGCCGCA		<i>Xho I</i>	His-Tag
ProGlnGlnGlyLeuArg / ⚡ / ProGlnLeuProLeuArgSerValAspLysLeuAlaAlaAlaLeuGlu		<u>CTCGAGC</u> CACCACCACCACCACCAC	STOP
		HisHisHisHisHisHisHis*	

⚡ Represents the site where the gene will be inserted.

Note: For correct expression, inserted gene needs to be in frame with pHTP2 5' gene sequence. Inserts correctly cloned into pHTP2 will maintain reading frames starting on the ATG codon.

2. Vector Sequence (5995 bp)

TGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAAGCGCGGGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCAGCGCCCTAGCGCCCGCTCCTTTCCGCTTTCTCCCT
 TCCTTTCTCGCCAGCTTCGCGCGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTAGGGTTCCGATTTAGTGTCTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTATG
 GTTACAGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCAAAATGGAACAACACTCAACCCATATCTCGGT
 CTATTTCTTTGATTATAAGGGATTTTCCCGATTTCGGCCATTGGTTAAAAATGAGCTGATTTAACAAAAATTAACCGGAATTTAACAAAAATATAACGTTTACAAATTCAGGT
 GGCACCTTTTCGGGAAATGTGCGCGGAACCCCTATTGTTTATTTTCTAAATACATTCAAAATATGATCCGCTCATGAATTAATTTAGAAAAACTCATCGAGCATCAAAATGAAAC
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 CGATTCGACCTCGTCCAACTATCAACAACCTATTAATTTCCCGCTCGTCAAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAAGATGGCAAAAGTTT
 ATGCAATTTCTTCCAGACTTGTTCACAGCCGACCCATTACGCTCGTATCAAAATCACTCGCATCAACCAACCGTTATTCATTCTGTGATTGCGCCTGAGCGAGACGAAATACCGGA
 TCCTGTTAAAAAGGACAATTACAACAGGAATCGAATGCAACCGCGCAGGAACTGCCAGGCATCAACAATATTTTACCTGAATCAGGATATCTTCTAATACCTGGAATGCTG
 TTTCCCGGGATCGCAGTGGTGAATACCATGCATCATCAGGAGTACGGATAAAATGCTTGTAGTGGTGGAAAGAGGCATAAAATCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGT
 AACATCATTTGGCAACGCTACCTTTGCCATGTTCCAGAAACAACCTTGGCGCATCGGGCTTCCATACAATCGATAGATTGTCGACCTGATTGCCCGACATTAATCGCGAGCCCATTTA
 TACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGCCCTAGAGCAAGACGTTTCCCGTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTA
 TTGTTTCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAAGCCCGTAGAAAAAGTCAAAGGATCTTCTTGTAGATCCTTTTTTCTGCGCGTAAATCTGTGCTTGC
 AAACAAAAAACCCCGCTACCAGCGGTGTTGTTTGGCGGATCAAGACTACCACTCTTTTCCGAAGTAACTGGCTTACGAGAGCGCAGATACCAATACTGCTCTTCTAGT
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 TAGTCTGTGCGGTTTCCGACCTTGTACTGTAGCGTCTGATTTTGTGATGCTCGTCAAGGGGGCGGAGCCTATGAAAAACCCAGCAACCGCGCCTTTTACGGTTCCTGGCCTTT
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 AGTCAAGTACGAGGAAAGCGGAAGAGCGCTGATGCGGTATTTTCTCTTACGCATCTGTGCGGTTTTCACACCGCATATATGGTGCCTCTCAGTACAATCTGCTGATGCGCGA
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 ATCCGGAACATAATGTTGACAGGCGCTGACTTCCCGCTTCCAGACTTACGAAACCGGAAACCGAAGACCATTATGTTGTTGCTCAGTTCGAGACGTTTTCGAGCAGCAGTCCG
 TACAGTTCCGCTGCGCTATCGGTTGATTCATCTGCTAACCAAGTACCGCAAGCCGCGGAGAGGCGTTAATGCTGGCTTCTGATAAAAGCGGGGCTTATTAAGCGGTTTTTCTCCTGTTTGGTCACTGATGCTC
 GCGGATAATGGCTGCTTCTCGCGAAACGTTTGGTGGCGGACAGTGCAGAAAGGCTTGAAGCGAGGGGCTGCAAGATTCCGAAATACCGCAAGCGACAGGCGCATCATCTGCGCCTC
 CAGCGAAAGCGGTTCTCGCGAAATGACCCAGAGCGCTGCGCGCACTGCTTACGAGTTGCAATGATAAAAGAGACAGTATAAGTGGCGCAGCAGTATGATGCCCGCGCCACC
 GGAAGGAGCTGACTGGTTGAAGGCTCTCAAGGCGATCGTTCGAGATCCCGGTCCTAATGAGTGAAGTAACTTACATTAATGCGTTGCGCTCACTGCCCGCTTCCAGTCCGGAAA
 CTTGCTGTCGCGCTGCAATTAATGAATCGGCCAACGCGCGGGGAGAGGCGTTAATGCTGGCTTCTGATAAAAGCGGGGCTTATTAAGCGGTTTTTCTCCTGTTTGGTCACTGATGCTC
 CACCGCTGCGCCTGAGAGAGTTGACGAAAGCGGTCACGCTGGTTTGGCCAGCAGGCAAAATCCTGTTTGTAGTGGTGAAGCGCGGATATAACATGAGCTGTCTTCGGTATCG
 TCGTATCCCACTACCGAGATATCCGCAACACGCGCAGCCCGACTCGGTAATGGCGGCATTTGCGCCAGCGCCATCTGATCGTTGGCAACAGCAGTCCGAGTGGGAACGATGCCCT
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 CGACGCGCGTGCAGGCGCAGACTGAGGTTGGCAACGCCAATCAGCAACGACTGTTTCCCGCGCAGTTGTTGTGCCAGCGGTTGGGAATGTAATTCAGCTCCGCCATCGCCGCTTCC
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 AGCCAGTAGTAGTTGAGCGGTTGAGCACCAGCCGCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCGCGCCAGGGGCTGCCACCATCCACGCCGAAACA
 AGCGCTCATGAGCCGAAGTGGCGAGCCGATCTTCCCATCGGTGATGTCGCGGATATAGCGCCAGCAACCGCACTGTGGCGCGGTTGATGCCGCCAGTATGCGGCTCGGCGTAG
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 CGTCCGACCGCAGTTGGCAGTGGATATTGCGGACCAATTACGCACTTGGCGTCCAGCTTGGGTTAGCGGTTACTCCGGCAGTTGTCTGAGCAATGGCACACTTGTTCGGGGTTAC
 CAGCGCGGAAAGAGATGAAAGAAATTTTCGACGAAACCAAAAAATGACCAGCGGTAAGGATCATCCATGGGACGACGCTCATCATCATCATCACAGCAGCGCCCTCAGCAAG
 GGCTGAGG/✂/CCTCAGCTTCCGCTGAGTCCGTCGACAAGCTTGGCGCGCACTCGAGCACCAACCAACCACTGAGATCCGGCTGCTAACAAAGCCGAAAGGAAAGCTGAG
 TTGGCTGCTGCCACCGTGAAGCAATAACTAGCATAAACCCCTTGGGGCTCTAAACGGGCTTGTAGGGGTTTTTTGCTGAAAGGAGGAATATATCCGGAT

pHP2 sequence landmarks:

- **T7 promoter:** in gray
- **First ATG (methionine):** in yellow
- **LLDsbc gene:** in green
- **His•Tag coding sequences:** in purple
- **Cloning region:** ✂
- **T7 terminator:** in dark gray
- **Sequencing primers (T7 universal and T7 terminator):** underlined
- **BglII, NcoI & XhoI recognition sites:** in bold

Sequence added to the final recombinant protein (25.98 kDa):

MGDDAAIQQTILAKMGIKSSDIQPAPVAGMKTVLNTNSGVLYITDDGKHI IQGPMYDVS GTAPVNVNTKMLLQLNALEKEMIVYKAPQEKHVI TVFTDITCGYC
 HKLHEQMADYNALGITVRYLAFPRQGLSDAEKEMKAIWCAKDKNKAFFDDVMAGKSVAPASCDVDIADHYALGVQLGVSGTPAVVLSNGTLVPGYQPPKEMKE
 FLDEHQKMTSGKGS SMGSSHHHHHSSSGPQQGLR