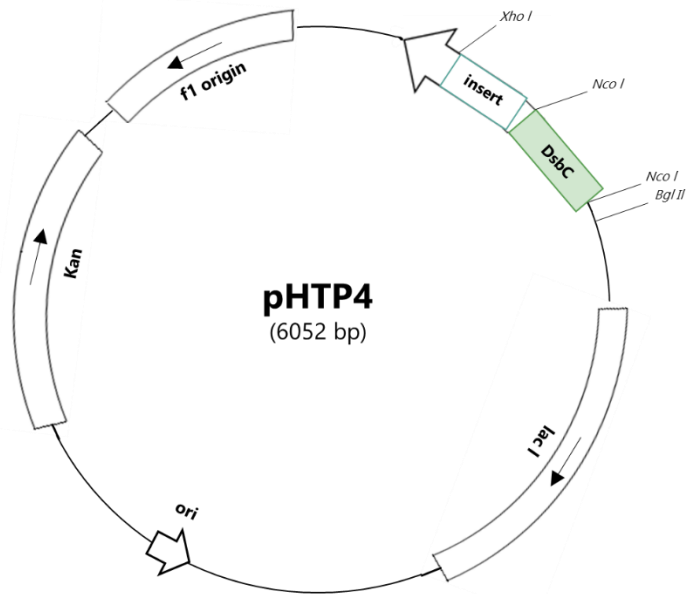


pHTP4 Vector

pHTP4 was designed for the cloning and expression of high-levels of recombinant proteins in *Escherichia coli*. Recombinant proteins are expressed in fusion with the disulfide-bond isomerase (DsbC), which is able to promote solubility and folding of disulfide bond-containing partners in the periplasm. This vector, included in the portfolio of NZYtech pHTP expression vectors, is part of the NZYEasy Cloning & Expression System. pHTP4 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



pHTP4 Cloning/Expression Region

<i>Nco I</i>	DsbC	<i>Nco I</i>	His-Tag
<u>CCATGG</u> GA AAGAAAGGTTTATGTTGTTTACT . 705bp . AAAATGACCAGCGGTAAGGATCAT		<u>CCATGG</u> GCAGCAGCCATCATCATCATCATCACAGCAGCGGC	
MetGlyLysLysGlyPheMetLeuPheThr . 235aa . LysMetThrSerGlyLysGlySerSerMetGlySerSer		HisHisHisHisHisHisHisSerSerGly	
CCTCAGCAAGGGCTGAGG / ⌘ / CCTCAGCTCCGCTGAGGTCCGTCGACAAGCTTGC GGCCGCA		<u>CTCGAGCACCACCACCACCACCAC</u> TGAGATCCGGCTGCT	
ProGlnGlnGlyLeuArg / ⌘ / ProGlnLeuProLeuArgSerValAspLysLeuAlaAlaAlaLeuGlu		HisHisHisHisHisHisHis*	STOP

⌘ Represents the site where the gene will be inserted.

Note: For correct expression, inserted gene needs to be in frame with pHTP4 5' gene sequence. Inserts correctly cloned into pHTP4 will maintain reading frames starting on the ATG codon.

2. Vector Sequence (6052 bp)

TTGCCAATGGGACGCGCCCTGTAGCGCGCATTAAAGCGCGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGGCCAGCGCCCTAGCGCCCGCTCTTTTCGCTTTCCCTTC
TCCTTTCTCGCCACGTTTCGCGCGGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCGGATTAGTGTCTTTACGGCACCTCGACCCCAAAAAAATTGATTAGGGTGTG
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CTATTCTTTGATTATAAGGGATTTGCGGATTTCCGGCTATTGGTTAAAAAATGAGCTGATTTAACAAAAAATTAACGGAAATTTAAACAAATATTAACGTTTACAATTTACAGGT
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CACCACCACCACCACCTGAGATCCGGCTGCTAACAAAGCCGAAAGGAAAGCTGAGTTGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCTCTAACCGGGTCT
TGAGGGGTTTTTTTCTGCTGAAAGGAGGAAGTATATCCGGAT

pHTP4 sequence landmarks:

- **T7 promoter:** in gray
- **First ATG (methionine):** in yellow
- **DsbC 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 (28.01 kDa):

MGKKGFMLFTLLAAFSGFAQDDAAIQQTLAKMGIKSSDIQPAPVAGMKTVLNSGVLYITDDGKHI IQGPMYDVSGTAPVNVNKNMLLKQ
LNALEKEMIVYKAPQEKHVI TVFTDITCGYCHKLHEQMDYNALGITVRYLAFPRQGLDSDAEKEMKAIWCAKDKNAFDDVMAGKSVAPA
SCDVDIADHYALGVQLVSGTAPAVVLSNGTLVPGYQPPKEMKEFLDEHQMTSGKSSMGSSHHHHHHSSGPOQGLR