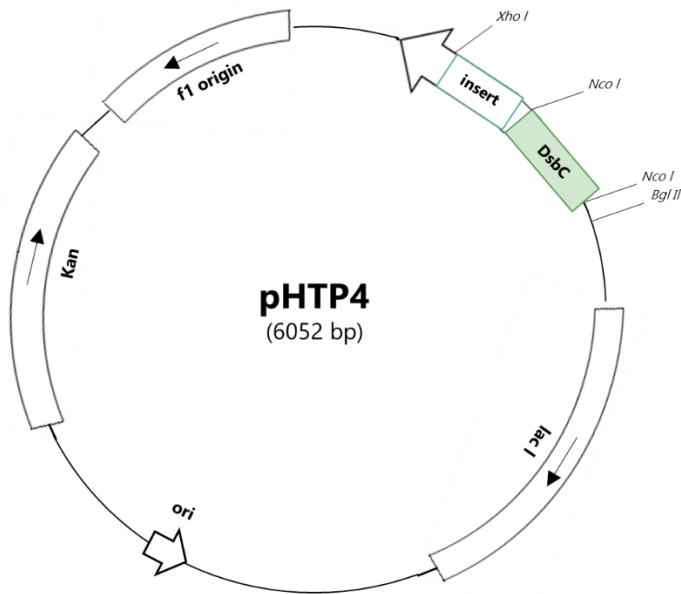


## pHTP4 Expression Vector

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>CCATGGG</u> AAAGAAAGGTTTTATGTTGTTTACT . 705bp . AAAATGACCAGCGGTAAAGGATCAT		<u>CCATGGG</u> GCAGCAGCCATCATCATCATCATCACAGCAGCGGC	
MetGlyLysLysGlyPheMetLeuPheThr . 235aa . LysMetThrSerGlyLysGlySerSerMetGlySerSer		HisHisHisHisHisHisHisSerSerGly	
CCTCAGCAAGGGCTGAGG / ⌘ / CCTCAGCTCCGCTGAGGTCGGTCGACAAGCTTGGCGGCCGCA		Xho I	His-Tag
ProGlnGlnGlyLeuArg / ⌘ / ProGlnLeuProLeuArgSerValAspLysLeuAlaAlaAlaLeuGlu		<u>CTCGAGC</u> ACCACCACCACCACCAC	STOP
		HisHisHisHisHisHisHis*	

⌘ 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)

TGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGCGGGTGTGGTGGTTACGCGCAGCGTACCCTGACACTTGCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCT  
TCCCTTCCTTTCTCGCCACGTTTCGCGGGTTCGCCCGTCAAGCTCAAATCGGGGGCTCCCTTAGGGTTCCGATTAGTGTCTTACGGCACCTCGACCCAAAAAAGTTGAT  
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CAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGGCGATTTCGGCTATTGGTTAAAAAATGAGCTGATTAACAAAAATTTAACCGGAATTTTAACAAAAATAT  
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CCCCGTAGAAAAGATCAAAGGATCTTTTGGATCCTTTTTTTTCGCGCTAATCTGCTGCTGCAAAAACAAAAAACCCCGCTACCAGCGGTGGTTTTGTTGCGCGATCAAG  
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GACAAGCTTGGCGCGCAC**CTCGGACCA**CCACCAC**CCAC**AGAGATCCGCTGCTAACAAGCCGAAAGGAGCTGAGTTGGCTGCTGCCACCCTGAGCAATA**ATAGC**  
**ATA**ACCCCTTGGGGCTTAAACGGGCTT**GAGGGGTTTTTTC**GCTGAAAGGAGAACTATATCCGGAT

### 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):**

MGKKGFMLFTLLAAFSGFAQADDAAIQQTLAKMGIKSSDIQPAPVAGMKTVLTNSGVLYITDDGKHI IQGPMYDVSGTAPVNVTKMLLKQLNALEKE  
MIVYKAPQEKHVITVFTDITCGYCHKLHEQMADYNALGITVRYLAFPRQGLSDAEKEMKAIWCAKDKNKAFDDVMAGKSVAPASCDVDIADHYALGV  
QLGVSGTPAVVLSNGTLVPGYQPPKEMKEFLDEHQKMTSGKGSSMGSSHHHHHSSGPQQGLR