

pKLAC1 Vector



1-800-632-7799
info@neb.com
www.neb.com



N3740S 001110813080

N3740S

20 µg Lot: 0011108 Exp: 8/13
1,000 µg/ml Store at -20°C

Description: The vector pKLAC1 directs high-level expression of a recombinant protein from the yeast *Kluyveromyces lactis* and is part of the *K. lactis* Protein Expression Kit (NEB #E1000). pKLAC1 can be used for either intracellular or secreted protein expression. SacII or BstXI linearized pKLAC1 integrates into the *LAC4* locus of the *K. lactis* genome upon transformation of *K. lactis* GG799 Competent Cells (NEB #C1001).

Vector pKLAC1 contains the strong *K. lactis* P_{LAC4-PBI} promoter (1), DNA encoding the *K. lactis*

α -mating factor (α MF) secretion domain (for secreted expression), a multiple cloning site (MCS), the *K. lactis* *LAC4* transcription terminator (TT), and a fungal acetamidase selectable marker gene (*amdS*) expressed from the yeast *ADH2* promoter (P_{ADH2}). An *E. coli* replication origin (*ori*) and ampicillin resistance gene (Ap^R) are present for propagation of pKLAC1 in *E. coli*.

NEB 5-alpha Competent *E. coli* (High Efficiency) (NEB #C2987), NEB 5-alpha Electrocompetent *E. coli* (NEB #C2989) and NEB 5-alpha Competent *E. coli* (Subcloning Efficiency) (NEB #C2988) are all recommended for propagation and subcloning this vector.

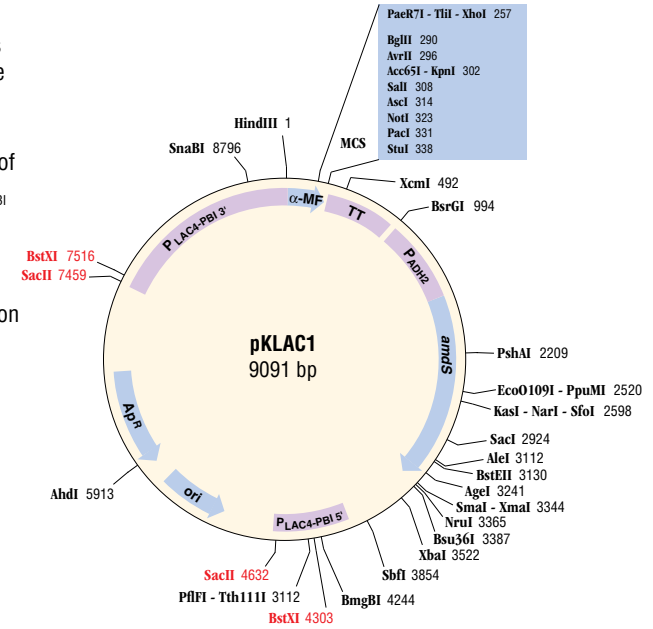
The sequence of the pKLAC1 vector (GenBank #AY968582) and additional pKLAC1 information are available at www.neb.com.

Source: pKLAC1 is isolated from *E. coli* strain ER2268 by a standard DNA purification procedure.

Supplied in: 10 mM Tris-HCl (pH 7.5), 1 mM EDTA .

Features of pKLAC1

- P_{LAC4-PBI} promoter does not express in *E. coli* allowing toxic genes to be cloned prior to their expression in yeast.
- Convenient MCS lies downstream of α MF secretion domain and P_{LAC4-PBI} promoter.
- Acetamidase expression for non-antibiotic selection in *K. lactis*.
- Ampicillin resistance for propagation in *E. coli*.



pKLAC1 plasmid map. Unique restriction sites are shown in bold. SacII and BstXI sites are shown in red.

(see other side)

CERTIFICATE OF ANALYSIS

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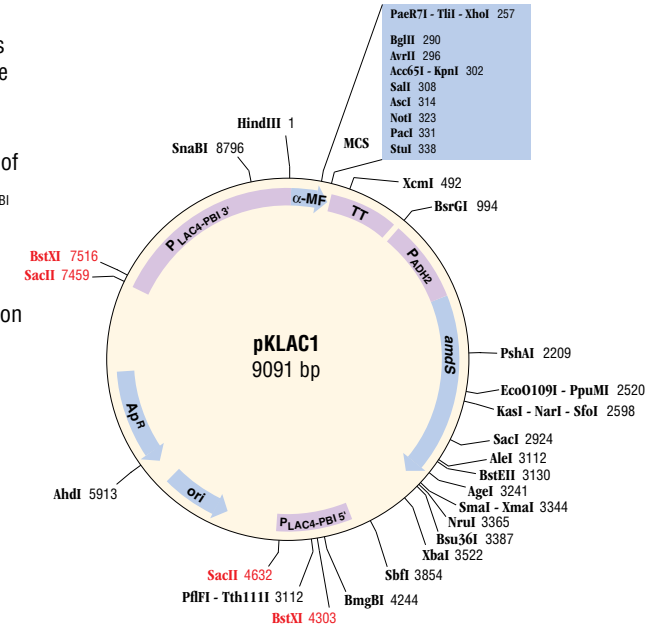
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(see other side)

CERTIFICATE OF ANALYSIS

8994 GAATTGTGAGCGGATAACAAGCTCAACACTTGA AATTTAGGAAAGAGCAGAATTTGGCAA 9053

Hind III

9054 AAAAAATAAAAAAAAAATAACACACATACTCATCGAGAAGCTTGAAAAAATGAAATTC 22
M K F

23 TCTACTATATTAGCCGCATCTACTGCTTTAATTTCCGTTGTTATGGCTGCTCCAGTTTCT 82
S T I L A A S T A L I S V V M A A P V S

83 ACCGAAACTGACATCGACGATCTTCCAATATCGGTTCCAGAAGAAGCCTTGATTGGATTC 142
T E T D I D D L P I S V P E E A L I G F

143 ATTGACTTAACCGGGGATGAAGTTTCCTTGTTCGCTGTTAATAACGGAACCCACACTGGT 202
I D L T G D E V S L L P V N N G T H T G

Xho I

203 ATTCTATTCTTAAACACCACCATCGCTGAAGCTGCTTTCGCTGACAAGGATGATCTCGAG 262
I L F L N T T I A E A A F A D K D D L E

Bgl II Avr II Kpn I Sal I Asc I

263 AAAAGAGAGGCTGAAGCTAGAAGCTAGATCTCCTAGGGGTACCGTCGACGGCGCGCCT 322
K R E A E A R R A R S P R G T V D G A P
↑ Kex cleavage

Not I Pac I Stu I

323 GCGGCCGCTTAATTAAGGCCTTGAATCGAGAATTTACTTAGATAAGTATGTACTTACA 382
A A A *

383 GGTATATTTCTATGAGATACTGATGTATACATGCATGATAATTTAAACGGTTATTAGT 442

443 GCCGATTGCTTGTGCGATAATGACGTTCTATCAAAGCAATACACTTACCACCTATTAC 502

pKLAC1 multiple cloning site (MCS). The *K. lactis* α -mating factor secretion domain is shown with a blue background. Only unique restriction sites are shown.

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8994 GAATTGTGAGCGGATAACAAGCTCAACACTTGA AATTTAGGAAAGAGCAGAATTTGGCAA 9053

Hind III

9054 AAAAAATAAAAAAAAAATAACACACATACTCATCGAGAAGCTTGAAAAAATGAAATTC 22
M K F

23 TCTACTATATTAGCCGCATCTACTGCTTTAATTTCCGTTGTTATGGCTGCTCCAGTTTCT 82
S T I L A A S T A L I S V V M A A P V S

83 ACCGAAACTGACATCGACGATCTTCCAATATCGGTTCCAGAAGAAGCCTTGATTGGATTC 142
T E T D I D D L P I S V P E E A L I G F

143 ATTGACTTAACCGGGGATGAAGTTTCCTTGTTCGCTGTTAATAACGGAACCCACACTGGT 202
I D L T G D E V S L L P V N N G T H T G

Xho I

203 ATTCTATTCTTAAACACCACCATCGCTGAAGCTGCTTTCGCTGACAAGGATGATCTCGAG 262
I L F L N T T I A E A A F A D K D D L E

Bgl II Avr II Kpn I Sal I Asc I

263 AAAAGAGAGGCTGAAGCTAGAAGCTAGATCTCCTAGGGGTACCGTCGACGGCGCGCCT 322
K R E A E A R R A R S P R G T V D G A P
↑ Kex cleavage

Not I Pac I Stu I

323 GCGGCCGCTTAATTAAGGCCTTGAATCGAGAATTTACTTAGATAAGTATGTACTTACA 382
A A A *

383 GGTATATTTCTATGAGATACTGATGTATACATGCATGATAATTTAAACGGTTATTAGT 442

443 GCCGATTGCTTGTGCGATAATGACGTTCTATCAAAGCAATACACTTACCACCTATTAC 502

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References:

- Colussi, P.A. and Taron, C.H. (2005) *Appl. Environ. Microbiol.*, 71, 7092–7098.

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