

pKLAC2 Vector



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



N3742S 001130715071

N3742S

20 µg Lot: **0011307** Exp: **7/15**
1,000 µg/ml Store at **-20°C**

Description: The vector pKLAC2 directs high-level expression of a recombinant protein from the yeast *Kluyveromyces lactis* and is compatible with the *K. lactis* Protein Expression Kit (NEB #E1000). pKLAC2 can be used for either intracellular or secreted protein expression. SacII or BstXI linearized pKLAC2 integrates into the *LAC4* locus of the *K. lactis* genome upon transformation of *K. lactis* competent cells. pKLAC2 differs from pKLAC1 (NEB #N3740) in that it contains a universal multiple cloning site (MCS) that is compatible with all NEB expression systems.

Vector pKLAC2 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 universal multiple cloning site (MCS), the *K. lactis* *LAC4* transcription terminator (TT), and a fungal acetamidase selectable marker gene (*amdS*) expressed from the yeast *ADH1* promoter (P_{ADH1}). An *E. coli* replication origin (*ori*) and ampicillin resistance gene (Ap_R) are present for propagation of pKLAC2 in *E. coli*.

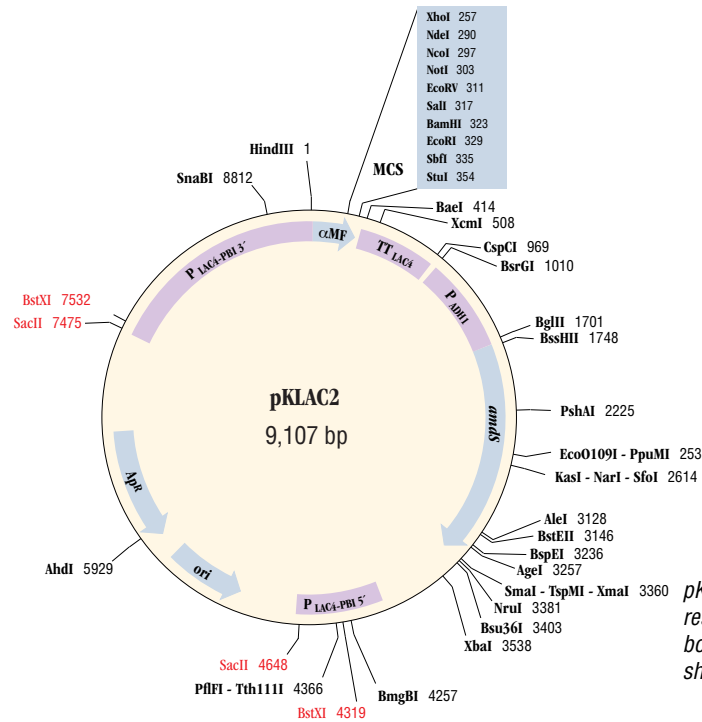
The sequence of the pKLAC2 vector (GenBank #EU196354) and additional pKLAC2 information are available at www.neb.com.

Source: pKLAC2 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 pKLAC2

- P_{LAC4-PBI} promoter does not express in *E. coli* allowing toxic genes to be cloned prior to their expression in yeast.
- Universal 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*.



pKLAC2 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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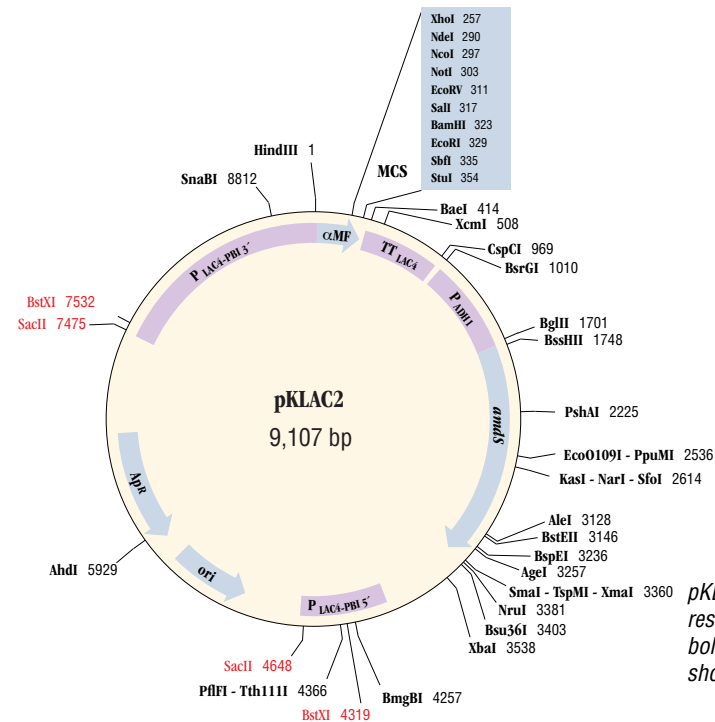
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CERTIFICATE OF ANALYSIS

9099 GAATTGTGAGCGGATAACAAGCTCAACACTTGAAATTTAGGAAAGAGCAGAATTTGGCAA 9068

HindIII

9069 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 ATTGACTTAACCGGGGATGAAGTTTCCTTGTTCCTGTTAATAACGGAACCCACTGGT 202
I D L T G D E V S L L P V N N G T H T G

XhoI

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

NdeI NcoI NotI EcoRV SalI

263 AAAAGAGAGGCTGAAGCTAGAAGAGCTCATATGTCATGGGCGGCCGATATCGTCGCAC 322
K R E A E A R R A H M S M G G R D I V D

BamHI EcoRI SbfI StuI

323 GGATCCGAATTCCTGCAGTAATTAATAAAGGCCCTTGAATCGAGAATTTATACTTAGA 382
G S E F P A G N *

383 TAAGTATGTACTTACAGGTATATTTCTATGAGATACTGATGTATACATGCATGATAATAT 442

443 TTAACGGTTATTAGTGCCGATTGTCTTGTGCGATAATGACGTTCTTATCAAAGCAATAC 502

*The K. lactis α-mating factor secretion domain is shown with a blue background.
Only unique restriction sites are shown.*

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9099 GAATTGTGAGCGGATAACAAGCTCAACACTTGAAATTTAGGAAAGAGCAGAATTTGGCAA 9068

HindIII

9069 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 ATTGACTTAACCGGGGATGAAGTTTCCTTGTTCCTGTTAATAACGGAACCCACTGGT 202
I D L T G D E V S L L P V N N G T H T G

XhoI

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

NdeI NcoI NotI EcoRV SalI

263 AAAAGAGAGGCTGAAGCTAGAAGAGCTCATATGTCATGGGCGGCCGATATCGTCGCAC 322
K R E A E A R R A H M S M G G R D I V D

BamHI EcoRI SbfI StuI

323 GGATCCGAATTCCTGCAGTAATTAATAAAGGCCCTTGAATCGAGAATTTATACTTAGA 382
G S E F P A G N *

383 TAAGTATGTACTTACAGGTATATTTCTATGAGATACTGATGTATACATGCATGATAATAT 442

443 TTAACGGTTATTAGTGCCGATTGTCTTGTGCGATAATGACGTTCTTATCAAAGCAATAC 502

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Usage Notes: 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.

References:

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

NOTICE TO BUYER/USER: The vector pKLAC2 is a component of an expression system that was developed from basic research at New England Biolabs, Inc. and DSM Biologics Company B.V. The buyer/user has a non-exclusive sublicense to use this system or any component thereof, including vector pKLAC2, for **RESEARCH PURPOSES ONLY**. A license to use this system for manufacture of clinical grade material or commercial purposes is available from New England Biolabs, Inc., or DSM Biologics Company B.V.

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