pKLMF-EK Vector

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

N3743S 001130915091

N3743S

20 µg **Lot: 0011309** **Exp: 9/15**

1,000 µg/ml **Store at –20°C**

Description: The vector pKLMF-EK directs high-level intracellular expression of a recombinant protein fused to the maltose binding protein (MBP) in the yeast *Kluyveromyces lactis*. It is compatible with the *K. lactis* Protein Expression Kit (NEB #E1000). MBP fusion proteins expressed from pKLMF-EK can be affinity purified from cell lysates using Amylose Resin (NEB #E8021). MBP can be removed from purified fusion proteins by digestion with Enterokinase (NEB #P8070).

Vector pKLMF-EK contains the strong *K. lactis* $P_{LAC4\text{-}PBI}$ promoter (1), DNA encoding *E. coli* maltose binding protein (MBP) with a C-terminal enterokinase site, 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) is present for propagation of pKLMF-EK in *E. coli*. SacII linearized pKLMF-EK integrates into the *LAC4* locus of the *K. lactis* genome upon transformation of *K. lactis* competent cells.

The sequence of the pKLMF-EK vector (GenBank #FJ010196) and additional pKLMF-EK information are available at www.neb.com.

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

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

Features of pKLMF-EK

- $P_{LAC4\text{-}PBI}$ promoter does not express in *E. coli* allowing toxic genes to be cloned prior to their expression in yeast.
- Universal MCS lies downstream of DNA encoding MBP and $P_{LAC4\text{-}PBI}$ promoter.
- Acetamidase expression for non-antibiotic selection in *K. lactis*.
- Ampicillin resistance for propagation in *E. coli*.

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                                                    HindIII
GAATTGTGAGCGGATAACAAGCTCAACACTTGAAATTTAGGAAAGAGCAGAATTTGGCAA
AAAAAATAAAAAAAAAATAAACACACATACTCATCGAGAAGCTTGCCACCATGAAAACTG
                                                  M  K  T

AAGAAGGTAAACTGGTAATCTGGATTAACGGCGATAAAGGCTATAACGGTCTCGCTGAAG
E  E  G  K  L  V  I  W  I  N  G  D  K  G  Y  N  G  L  A  E

TCGGTAAGAAATTCGAGAAAGATACCGGAATTAAAGTCACCGTTGAGCATCCGGATAAAC
V  G  K  K  F  E  K  D  T  G  I  K  V  T  V  E  H  P  D  K

TGGAAGAGAAATTCCCACAGGTTGCGGCAACTGGCGATGGCCCTGACATTATCTTCTGGG
L  E  E  K  F  P  Q  V  A  A  T  G  D  G  P  D  I  I  F  W

CACACGACCGCTTTGGTGGCTACGCTCAATCTGGCCTGTTGGCTGAAATCACCCCGGACA
A  H  D  R  F  G  G  Y  A  Q  S  G  L  L  A  E  I  T  P  D

AAGCGTTCCAGGACAAGCTGTATCCGTTTACCTGGGATGCCGTACGTTACAACGGCAAGC
K  A  F  Q  D  K  L  Y  P  F  T  W  D  A  V  R  Y  N  G  K

TGATTGCTTACCCGATCGCTGTTGAAGCGTTATCGCTGATTTATAACAAAGATCTGCTGC
L  I  A  Y  P  I  A  V  E  A  L  S  L  I  Y  N  K  D  L  L

CGAACCCGCCAAAAACCTGGGAAGAGATCCCGGCGCTGGATAAAGAACTGAAAGCGAAAG
P  N  P  P  K  T  W  E  E  I  P  A  L  D  K  E  L  K  A  K

GTAAGAGCGCGCTGATGTTCAACCTGCAAGAACCGTACTTCACCTGGCCGCTGATTGCTG
G  K  S  A  L  M  F  N  L  Q  E  P  Y  F  T  W  P  L  I  A

CTGACGGGGGTTATGCGTTCAAGTATGAAAACGGCAAGTACGACATTAAAGACGTGGGCG
A  D  G  G  Y  A  F  K  Y  E  N  G  K  Y  D  I  K  D  V  G

TGGATAACGCTGGCGCGAAAGCGGGTCTGACCTTCCTGGTTGACCTGATTAAAAACAAAC
V  D  N  A  G  A  K  A  G  L  T  F  L  V  D  L  I  K  N  K

ACATGAATGCAGACACCGATTACTCCATCGCAGAAGCTGCCTTTAATAAAGGCGAAACAG
H  M  N  A  D  T  D  Y  S  I  A  E  A  A  F  N  K  G  E  T

CGATGACCATCAACGGCCCGTGGGCATGGTCCAACATCGACACCAGCAAAGTGAATTATG
A  M  T  I  N  G  P  W  A  W  S  N  I  D  T  S  K  V  N  Y

GTGTAACGGTACTGCCGACCTTCAAGGGTCAACCATCCAAACCGTTCGTTGGCGTGCTGA
G  V  T  V  L  P  T  F  K  G  Q  P  S  K  P  F  V  G  V  L

GCGCAGGTATTAACGCCGCCAGTCCGAACAAAGAGCTGGCAAAAGAGTTCCTCGAAAACT
S  A  G  I  N  A  A  S  P  N  K  E  L  A  K  E  F  L  E  N

ATCTGCTGACTGATGAAGGTCTGGAAGCGGTTAATAAAGACAAACCGCTGGGTGCCGTAG
Y  L  L  T  D  E  G  L  E  A  V  N  K  D  K  P  L  G  A  V

CGCTGAAGTCTTACGAGGAAGAGTTGGCGAAAGATCCACGTATTGCCGCCACTATGGAAA
A  L  K  S  Y  E  E  E  L  A  K  D  P  R  I  A  A  T  M  E

ACGCCCAGAAAGGTGAAATCATGCCGAACATCCCGCAGATGTCCGCTTTCTGGTATGCCG
N  A  Q  K  G  E  I  M  P  N  I  P  Q  M  S  A  F  W  Y  A

TGCGTACTGCGGTGATCAACGCCGCCAGCGGTCGTCAGACTGTCGATGAAGCCCTGAAAG
V  R  T  A  V  I  N  A  A  S  G  R  Q  T  V  D  E  A  L  K

                                  enterokinase site      XhoI
ACGCGCAGACTAATTCGAGCTCGGATGACGATGACAAGCTCGAGAAAAGAGAGGCTGAAG
D  A  Q  T  N  S  S  S  D  D  D  D  K  L  E  K  R  E  A  E

                  NdeI     NcoI     NotI     EcoRV    SalI     BamHI    EcoRI    SbfI
CTAGAAGAGCTCATATGTCCATGGGCGGCCGCGATATCGTCGACGGATCCGAATTCCCTG
A  R  R  A  H  M  S  M  G  G  R  D  I  V  D  G  S  E  F  P

            StuI
CAGGTAATTAAATAAAGGCCTTGAATCGAGAATTTATACTTAGATAAGTATGTACTTACA
A  G  N  *
GGTATATTTCTATGAGATACTGATGTATACATGCATGATAATATTTAAACGGTTATTAGT


GCCGATTGTCTTGTGCGATAATGACGTTCCTATCAAAGCAATACACTTACCACCTATTAC
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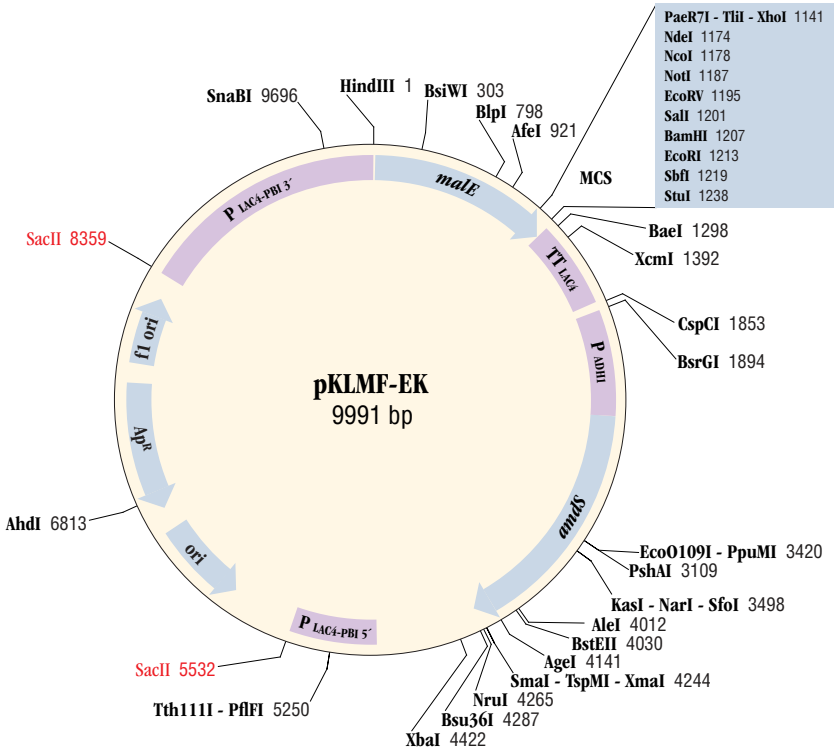

pKLMF-EK plasmid map. Unique restriction sites are shown in bold. SacII sites are shown in red.

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

Usage Notes: For proper integration into the LAC4 promoter region of the *K. lactis* chromosome, pKLMF-series vectors containing a gene of interest must be linearized with SacII prior to their introduction into *K. lactis* cells. The cloned gene of interest must have no internal SacII sites.

After transformation of *K. lactis* cells by a pKLMF-series vector, its targeted integration into the LAC4 promoter locus can be confirmed by whole-cell PCR using an adaptation of Optional Methods I and II of the *K. lactis* Protein Expression Kit Instruction Manual (#E1000). In these methods, Integration Primer 2 is a reverse primer that anneals to a specific region of DNA encoding the α–mating factor secretion leader sequence in pKLAC-series vectors, a region that is absent from pKLMF-series vectors. For assessment of the integration patterns of pKLMF-series vectors, a primer with the sequence 5´ d(GTTTACCTTCTTCAGTTTTCAT) 3´ should be used in place of the kit's Integration Primer 2 in these protocols (2). Whole cell PCR using this primer in conjunction with either Integration Primer 1 or Integration Primer 3 will each yield a 2.3 kb diagnostic amplicon.

(see other side)

CERTIFICATE OF ANALYSIS

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:

1. Colussi, P.A. and Taron, C.H. (2005) *Appl. Environ. Microbiol.*, 71, 7092–7098.
2. Foster, J. et al. (2008) *Parasitol. Res.* In press.