

pKLMF-EK Vector



N3743S

20 µg **Lot: 0011210** **Exp: 10/14**
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-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.

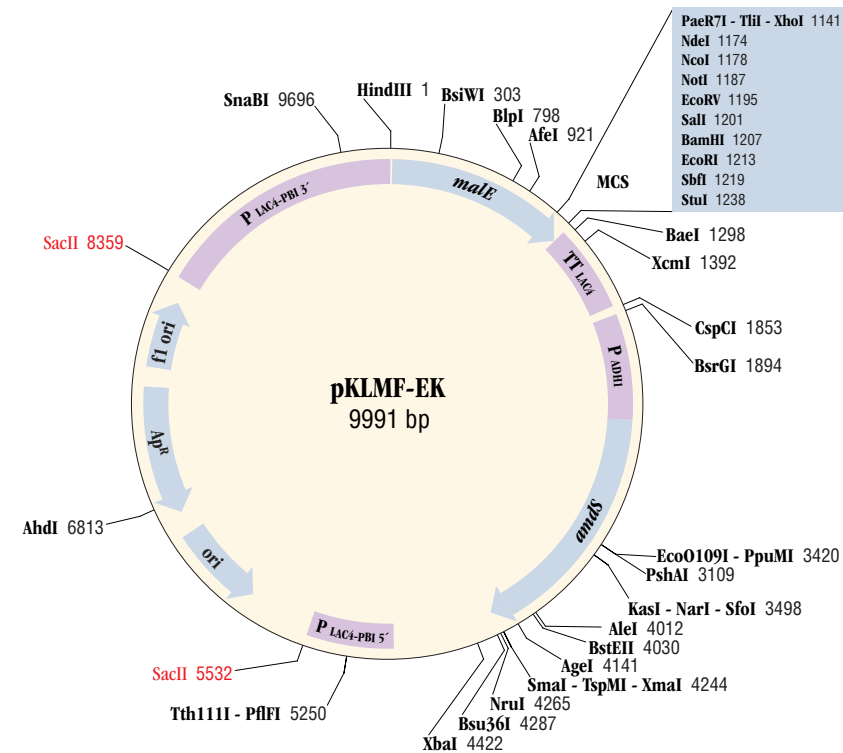
Source: pKLMF-EK is isolated from *E. coli* strain ER2268 by a standard DNA purification procedure.

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

Features of pKLMF-EK

- P_{LAC4-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-PBI} promoter.
- Acetamidase expression for non-antibiotic selection in *K. lactis*.
- Ampicillin resistance for propagation in *E. coli*.

```
GAATTGTGAGCGGATAACAAGCTCAACACTTGAATTTAGGAAAAGCAGAATTTGGCAA
                                     HindIII
AAAAATAAAAAAAAAATAACACACATACTCATCGAGAAGCTTGCCACCATGAAAACCTG
                                     M K T
AAGAAGGTAACCTGGTAATCTGGATTAACGGCGATAAAGGCTATAACGGTCTCGTGAAG
E E G K L V I W I N G D K G Y N G L A E
TCGGTAAGAAATTCGAGAAAGATACCGGAATTAAGCTCACCGTTGAGCATCCGGATAAAC
V G K K F E K D T G I K V T V E H P D K
TGGAGAGAAATCCCACAGGTTGCGCAACTGGCGATGGCCCTGACATTATCTTCTGGG
L E E K F P Q V A A T G D G P D I I F W
CACACGACCCTTTGGTGGCTACGCTCAATCTGGCTGTTGGCTGAAATCACCCGGACA
A H D R F G G Y A Q S G L L A E I T P D
AAGCGTTCAGGACAAGCTGTATCCGTTTACCTGGGATGCCGTACGTTACAACGGCAAGC
K A F Q D K L Y P F T W D A V R Y N G K
TGATTGCTTACCGATCGCTGTTGAAGCGTTATCGCTGATTATAACAAAGATCTGCTGC
L I A Y P I A V E A L S L I Y N K D L L
CGAACCCGCAAAAACTGGGAAGAGATCCCGCGCTGGATAAAGAACTGAAAGCGAAAG
P N P P K T W E E I P A L D K E L K A K
GTAAGAGCGCGTGTGTTCAACCTGCAAGAACCGTACTTACCTGGCCGCTGATTGCTG
G K S A L M F N L Q E P Y F T W P L I A
CTGACGGGGTTATGCGTTCAAGTATGAAACGGCAAGTACGACATTAAGAGCTGGGCG
A D G G Y A F K Y E N G K Y D I K D V G
TGGATAACGCTGGCGCAAGCGGGTCTGACCTTCTGGTTGACCTGATTAACAAACAAAC
V D N A G A K A G L T F L V D L I K N K
ACATGAATGCAGACACCGATTACTCCATCGCAGAAGCTGCCTTTAATAAAGCGAAACAG
H M N A D T D Y S I A E A A F N K G E T
CGATGACCATCAACGGCCGTTGGGCATGGTCCAACATCGACACAGCAAAGTGAATTATG
A M T I N G P W A W S N I D T S K V N Y
GTGTAACGGTACTGCCGACCTTCAAGGGTCAACCATCCAAACCGTTGTTGGCGTCTGA
G V T V L P T F K G Q P S K P F V G V L
GCGCAGGTATTAACGCCGCCAGTCCGAACAAAGAGCTGGCAAAAGAGTTCTCGAAAAC
S A G I N A A S P N K E L A K E F L E N
ATCTGCTGACTGATGAAGTCTGGAAGCGGTTAATAAAGACAACCGCTGGTGGCCTAG
Y L L T D E G L E A V N K D K P L G A V
CGTGAAAGTCTTACGAGGAAGAGTTGGCGAAAGATCCACGATGTCGCCACTATGGAAA
A L K S Y E E E L A K D P R I A A T M E
ACGCCAGAAAGGTGAAATCATGCCGAACATCCCGCAGATGTCGCTTCTGGTATGCCG
N A Q K G E I M P N I P Q M S A F W Y A
TGGTACTGCGGTGATCAACGCCGCCAGCGGTGCTGAGACTGTCGATGAAGCCCTGAAAG
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 Sall BamHI EcoRI SbfI
CTAGAAGAGCTCATATGTCATGGCGGCGCGATATCGTCGACGGATCCGAATCCCTG
A R R A H M S M G G R D I V D G S E F P
                                     StuI
CAGGTAATTAATAAAGGCCCTTGAATCGAAGATTTATACTTAGATAAGTATGACTTACA
A G N *
GGTATATTTCTATGAGATACTGATGTATACATGCATGATAATATTTAAACGGTTATTAGT
GCCGATTGCTGTGCGATAATGACGTTCTCATCAAGCAATACACTACCACCTATTAC
```



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

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(GTTTACCTTCTCAGTTTTCAT) 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.

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.

(see other side)

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.

NOTICE TO BUYER/USER: The vector pKLMF-EK 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 pKLMF-EK, 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.

U.S. Patent No. 5,643,758