



New England Biolabs, Inc.  
Tel: 800-632-5227 (orders)  
Tel: 800-632-7799 (support)  
Fax: 978-921-1350  
info@neb.com  
www.neb.com

## K. lactis Protein Expression Kit

#E1000S .....\$595 (USA)

### Description

The *K. lactis* Expression Kit provides an easy method for expressing a gene of interest in the yeast *Kluyveromyces lactis* (Figure 1). Proteins may be produced intracellularly or be secreted using the supplied integrative expression vector pKLAC1 (Figure 3). To achieve protein secretion, a gene of interest is cloned downstream of the *K. lactis*  $\alpha$ -mating factor secretion domain ( $\alpha$ -MF; Figure 4) which is eventually processed in the Golgi resulting in secretion of the desired protein (Figure 1).

The *K. lactis* expression system offers several advantages over other yeast and bacterial protein expression systems. First, *K. lactis* has been used to produce proteins at industrial scale in the food industry for over a decade due to its ability to rapidly achieve high culture densities and abundantly produce recombinant proteins (Figure 2). Second, yeast expression is driven by a variant of the strong *LAC4* promoter that has been modified to lack background expression in *E. coli* (1). Therefore, genes toxic to *E. coli* can be cloned into pKLAC1 in bacteria prior to their expression in yeast. Third, the kit includes highly competent *K. lactis* cells making the technology easy-to-use for those not accustomed to working with yeast. Their high transformation efficiency makes the system suitable for methods that require large numbers of transformants, for example, expression cloning using cDNA libraries. Selection of yeast transformants uses a unique antibiotic-free method in which acetamidase (*amdS*) expressed from pKLAC1 permits transformed cells to utilize acetamide as a sole nitrogen source on defined medium. Acetamide selection promotes formation of cells containing multiple integrations of pKLAC1 which results in higher yields of protein. Finally, proteins expressed in *K. lactis* have access to eukaryotic protein folding and glycosylation machinery that *E. coli* cells do not possess, making it an important alternative to bacterial expression systems.

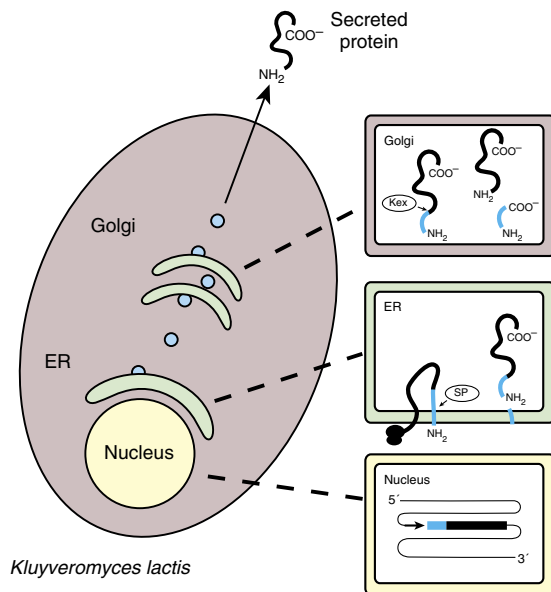
### Advantages

- High yield protein expression
- Rapid high cell density growth
- Clone and express genes toxic to *E. coli*
- Supplied with competent *K. lactis* cells
- No expensive antibiotics or methanol required
- Easy-to-use protocols for those inexperienced with yeast systems
- Attractive commercial sublicensing

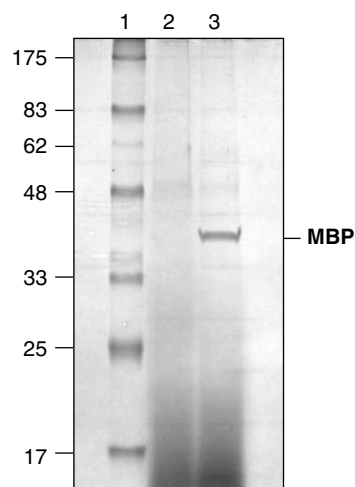
### The *K. lactis* Protein Expression Kit Includes:

pKLAC1 and pKLAC1-*malE* (20  $\mu$ g) Vectors  
Sac II (2,000 units)  
Integration Identification Primers  
*K. lactis* Competent Cells and Transformation Reagent  
Selective Yeast Media  
Instruction Manual

Technical Bulletins  
*K. lactis* (8/17/05)



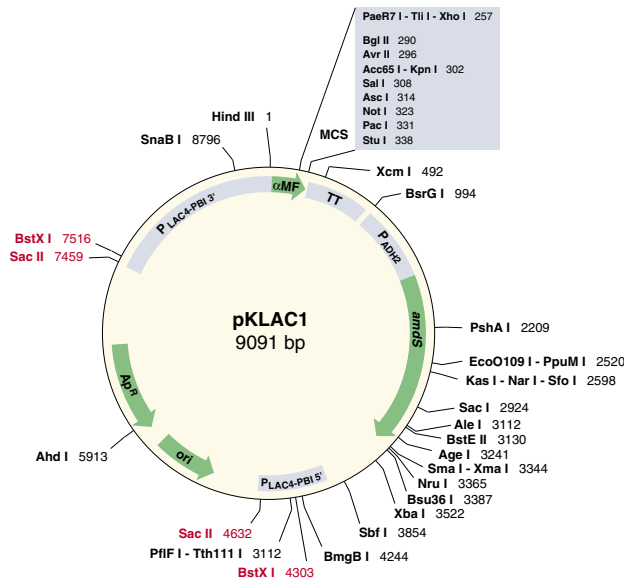
**Figure 1:** Secreted protein processing. In the nucleus, an integrated expression vector encoding a fusion between the  $\alpha$ -MF domain (blue) and a desired protein (black) is expressed. A signal peptide in the  $\alpha$ -MF domain directs entry of the fusion protein into the endoplasmic reticulum (ER) and is removed by signal peptidase (SP). The fusion protein is transported to the Golgi where the Kex protease removes the  $\alpha$ -MF domain. The protein of interest is then secreted from the cell.



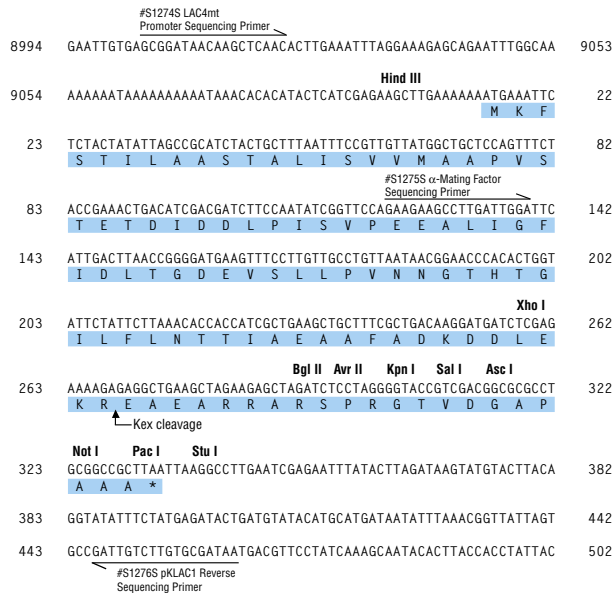
**Figure 2:** SDS-polyacrylamide gel electrophoresis separation of secreted recombinant maltose binding protein (MBP) and detection by Coomassie staining. Lane 1: Protein Molecular Weight Markers. Lane 2: spent culture medium (15  $\mu$ l) from wild-type *K. lactis* cells. Lane 3: spent culture medium (15  $\mu$ l) from *K. lactis* cells harboring an integrated expression cassette containing the *E. coli malE* gene.

(See other side)

***K. lactis* Protein Expression Kit (continued)**



**Figure 3:** The pKLAC1 expression vector. pKLAC1 (9091 bp) contains the 5' and 3' ends of the LAC4 promoter ( $P_{LAC4-PBI}$ ) separated by DNA encoding  $\beta$ -lactamase ( $Ap^R$ ) and the pMB1 origin ( $ori$ ) to allow for its propagation in *E. coli*. The *K. lactis*  $\alpha$ -mating factor secretion leader sequence ( $\alpha$ -MF), multiple cloning site (MCS), and the LAC4 transcription terminator (TT) lie immediately downstream of 3'  $P_{LAC4-PBI}$ . The yeast ADH2 promoter ( $P_{ADH2}$ ) drives expression of an acetamidase selectable marker gene ( $amdS$ ). The vector can be linearized by digestion with Sac II or BstX I to create a linear DNA fragment capable of inserting into the native LAC4 promoter region of the *K. lactis* genome.



**Figure 4:** pKLAC1 multiple cloning site. pKLAC1 (9091 bp) contains the *K. lactis*  $\alpha$ -mating factor secretion leader sequence (blue background) and a polylinker immediately downstream of the  $P_{LAC4-PBI}$  promoter. Unique polylinker restriction sites are indicated. Half-arrows show the positions of pKLAC1-specific sequencing primers available from New England Biolabs.

**References**

1. Colussi, P.A. and Taron, C.H. unpublished observations.

**Kit Components Sold Separately**

pKLAC1 Vector #N3740S	20 $\mu$ g	.....	\$295 (USA)
pKLAC1- <i>malE</i> Vector #N3741S	20 $\mu$ g	.....	available soon
Sac II #R0157S	2,000 units	.....	\$53 (USA)
#R0157L	10,000 units	.....	\$212 (USA)
Integration Primer 1 #N1277S	500 $\mu$ l	.....	available soon
Integration Primer 2 #N1278S	1,000 $\mu$ l	.....	available soon
Integration Primer 3 #N1279S	500 $\mu$ l	.....	available soon
<i>K. lactis</i> GG799 Competent Cells #C1001S	5 reactions	.....	\$75 (USA)

**Companion Products**

BstX I #R0113S	1,000 units	.....	\$58 (USA)
#R0113L	5,000 units	.....	\$232 (USA)
LAC4mt Promoter Sequencing Primer #S1274S	0.5 $A_{260}$ units	.....	\$100 (USA)
$\alpha$ -Mating Factor Sequencing Primer #S1275S	0.5 $A_{260}$ units	.....	\$100 (USA)
pKLAC1 Reverse Sequencing Primer #S1276S	0.5 $A_{260}$ units	.....	\$100 (USA)
<i>Taq</i> DNA Polymerase with ThermoPol Buffer #M0267S	400 units	.....	\$50 (USA)
#M0267L	2,000 units	.....	\$200 (USA)
<i>Taq</i> DNA Polymerase with Standard Buffer #M0273S	400 units	.....	\$50 (USA)
#M0273L	2,000 units	.....	\$200 (USA)
Deoxynucleotide Solution Mix #N0447S	8 $\mu$ mol of each	.....	\$63 (USA)
#N0447L	40 $\mu$ mol of each	.....	\$252 (USA)

**Notice to Buyer/User:** The *K. lactis* Protein Expression Kit was developed from basic research at New England Biolabs, Inc. and DSM Biologics Company B.V. The buyer and user has a non-exclusive sublicense to use this system or any component thereof, including the *K. lactis* GG799 Competent Cells, 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.