

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* α -mating factor secretion domain (α -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 μ g) Vectors
 Sac II (2,000 units)
 Integration Identification Primers
K. lactis Competent Cells and Transformation Reagent
 Selective Yeast Media
 Instruction Manual

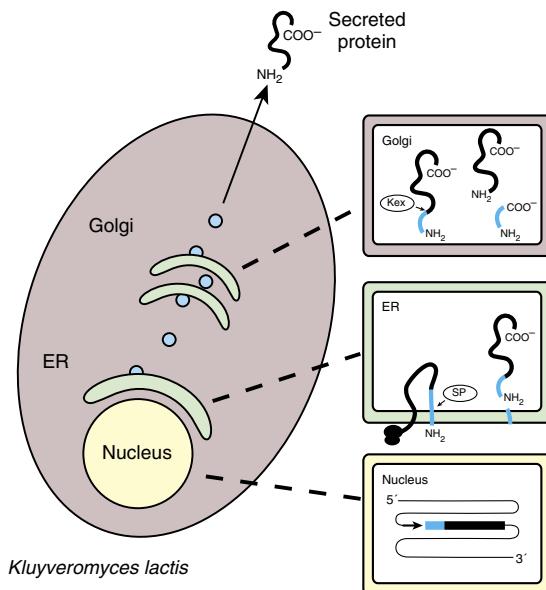


Figure 1: Secreted protein processing. In the nucleus, an integrated expression vector encoding a fusion between the α -MF domain (blue) and a desired protein (black) is expressed. A signal peptide in the α -MF domain directs entry of the fusion protein into the endoplasmic reticulum (ER) and is removed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

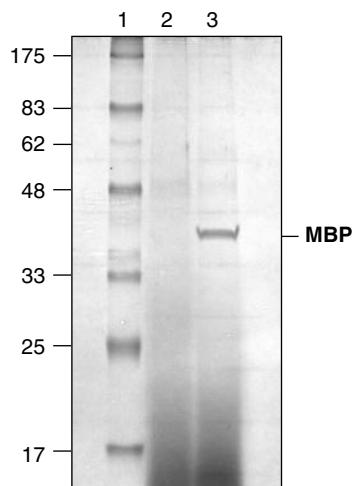


Figure 2: SDS-polyacrylamide gel electrophoresis separation of secreted recombinant maltose binding protein (MBP) and detection by Coomassie staining. Lane 1: Protein Molecular Weight Markers. Lane 2: spent culture medium (15 μ l) from wild-type *K. lactis* cells. Lane 3: spent culture medium (15 μ l)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

(See other side)

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

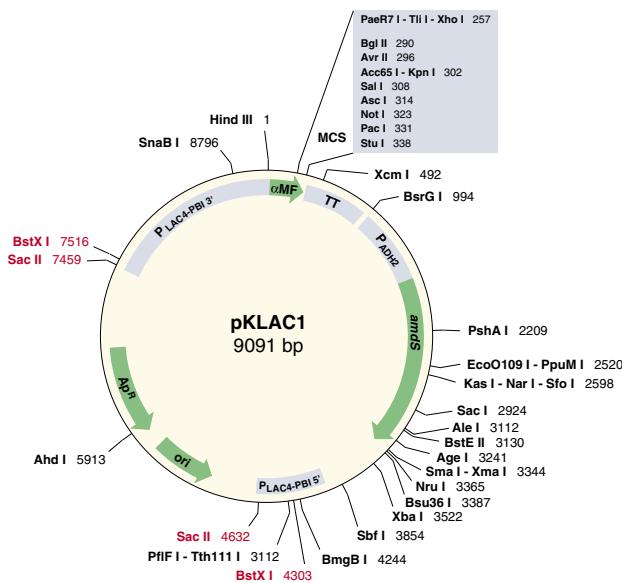


Figure 3: The *pKLAC1* expression vector. *pKLAC1* (9091 bp) contains the 5' and 3' ends of the *LAC4* promoter ($P_{LAC4-PB1}$) separated by DNA encoding β -lactamase (Ap^R) and the *pMB1* origin (*ori*) to allow for its propagation in *E. coli*. The *K. lactis* α -mating factor secretion leader sequence (α -MF), multiple cloning site (MCS), and the *LAC4* transcription terminator (TT) lie immediately downstream of 3' P_{LAC4-PB1}. 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 *Bst*X I to create a linear DNA fragment capable of inserting into the native *LAC4* promoter region of the *K. lactis* genome.

#S1274S LAC4mt Promoter Sequencing Primer									
8994	GAATTGTGAGCGGATAACAAGCTCACACTGAAATTAGGAAAGAGCAGAATTGGCAA								9053
	Hind III								
9054	AAAAAATAAAAAAATAAACACATACTCATGAGAGCTGTTAGGAAATTAAGGAAATTC								22
	M K F								
23	TCTACTATATTAGCCGCATCTACTGCTTAATTCCGTTTATGGCTGCTCAGTTCT								82
	S	T	I	L	A	A	S	T	
83	#S1275S α -Mating Factor Sequencing Primer								
	ACCGAAACTGACATCGACGATCTTCAATATCGGTTCCAGAAAGACCTGATTGGATTC								142
	T	E	T	D	I	D	D	L	
143	ATTGACTTAACCGGGGATGAAGTTCTCTGTTGCTGTTAACCGGAAACCCACACTGGT								202
	I	D	L	T	G	D	E	V	
	Xba I								
203	ATTCTATCTAAACACACCATCGCTGAAGCTGCTTCGCTGACAAGGATGATCTCGAG								262
	I	L	F	L	N	T	T	I	
	Bgl II								
263	AAAAGAGGGCTGAAGCTAGAACAGCTAGATCTCTAGGGGCTACCGTCGAGCGGCCCT								322
	K	R	E	A	E	A	R	R	
	Avr II								
	Kpn I								
	Sal I								
	Asc I								
	↑ Kex cleavage								
323	Not I								382
	Pac I	Pac I		Stu I					
383	GCGCCCTCTTAATTAGGCTCTGAATCGAGAATTATACTTAGATAAGTATGACTTACA								442
443	#S1276S pLAC1 Reverse Sequencing Primer								502
	A	A	A	*					

Figure 4: *pKLAC1* multiple cloning site. *pKLAC1* (9091 bp) contains the *K. lactis* α -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 µg	\$295 (USA)
pKLAC1- <i>malE</i> Vector #N3741S	20 µg	available soon
Sac II		
#R0157S	2,000 units	\$53 (USA)
#R0157L	10,000 units	\$212 (USA)
Integration Primer 1 #N1277S	500 µl	available soon
Integration Primer 2 #N1278S	1,000 µl	available soon
Integration Primer 3 #N1279S	500 µl	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)
α-Mating Factor Sequencing Primer			
#S1275S	0.5 A ₂₆₀ units	\$100 (USA)
pKLAC1 Reverse Sequencing Primer			
#S1276S	0.5 A ₂₆₀ units	\$100 (USA)
Taq DNA Polymerase with ThermoPol Buffer			
#M0267S	400 units	\$50 (USA)
#M0267L	2,000 units	\$200 (USA)
Taq DNA Polymerase with Standard Buffer			
#M0273S	400 units	\$50 (USA)
#M0273L	2,000 units	\$200 (USA)
Deoxynucleotide Solution Mix			
#N0447S	8 μmol of each	\$63 (USA)
#N0447L	40 μmol of each	\$252 (USA)

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