Choose the kit or vector that's right for you

You can put the power of *P. pastoris* into your laboratory with a complete *Pichia* Expression Kit or with individual *Pichia* expression vectors. Three *Pichia* Expression Kits are available, each of which includes vectors, *P. pastoris* strains, reagents for transformation, sequencing primers, media, and a comprehensive manual (Table 3). In addition, the pPICZ, pGAPZ, and pPIC6 vectors are available separately (see insert for ordering information). Choose the kit or vector that best meets your research needs and take advantage of the proven strength of *P. pastoris*.

Table 3—*Pichia* expression kit components.

	EasySelect™ *Pichia* Expression Kit	Multi-Copy *Pichia* Expression Kit	Original *Pichia* Expression Kit
Vectors	pPICZ A, B, C (20 µg each) pPICZα A, B, C (20 µg each)	pPIC9K (20 µg) pPIC3.5K (20 µg) pAO815 (20 µg)	pPIC9 (10 µg) pPIC3.5 (10 µg) pHIL-D2 (10 µg) pHIL-S1 (10 µg)
Strains	X-33 (Mut$^+$, His$^+$) GS115 (Mut$^+$) KM71 (Mut^S) GS115/pPICZ/*lac*Z (control)	GS115 (Mut$^+$) KM71 (Mut^S) GS115/β-gal (control) GS115/albumin (control)	GS115 (Mut$^+$) KM71 (Mut^S) GS115/β-gal (control) GS115/albumin (control)
Transformation reagents	*Pichia* EasyComp™ Kit (120 transformations)	Spheroplast Module (50 transformations)	Spheroplast Module (50 transformations)
Sequencing primers	5′ *AOX1* 3′ *AOX1* α-factor	5′ *AOX1* 3′ *AOX1* α-factor	5′ *AOX1* 3′ *AOX1* α-factor
Media and supplements	YP base medium (2 pouches)* YP base agar (2 pouches)* Yeast Nitrogen Base (1 pouch)† Zeocin™ (250 mg)	YP base medium (2 pouches)* YP base agar (2 pouches)* Yeast Nitrogen Base (1 pouch)†	YP base medium (2 pouches)* YP base agar (2 pouches)* Yeast Nitrogen Base (1 pouch)†

* Each pouch contains reagents to prepare 1 L of medium.

† Each pouch contains reagents to prepare 500 ml of 10X YNB.

The power of *Pichia* in your lab

Make the most out of your protein expression. Put the power of *P. pastoris* into your laboratory and experience the advantages of a high-yield, easy-to-use, proven expression system. See the Ordering Information insert, then call Invitrogen and order today.

References:

1. Cregg, J.M. et al. (1993) *Bio/Technology* 11: 905–910.

2. Waterham, H.R. et al. (1997) *Gene* 186: 37–44.

Put the proven strength of *Pichia* behind your protein expression

Pichia pastoris Expression System

invitrogen™

The expression system for ease, yield, and functionality

Pichia pastoris Expression System

- → High yields—up to grams of protein per liter; scalable to 10,000 L fermentors
- → Easy-to-use methods—as easy as bacterial systems
- → Proven record—numerous literature citations available

Some expression systems are easy to use and produce high yields, but the protein is inactive. Others produce functional protein, but are difficult, time-consuming, and low-yielding. Either way, your expression experiment is not giving you what you need. Find the best of both worlds in the *Pichia pastoris* Expression System. Three key factors make *Pichia* a desirable host: high biomass—cell density can be ten times greater than that of *Saccharomyces cerevisiae* cultures (Figure 1)—increases production; strong promoters drive high levels of expression; and efficient protein secretion signals simplify purification. Together, these features provide you with high yields of functional protein in an easy-to-manage, cost-effective microbial host.

How it works

The host for high-level recombinant protein expression in the *Pichia* Expression System is the methylotrophic yeast *Pichia pastoris*. In the absence of glucose, *P. pastoris* uses methanol as a carbon source. The alcohol oxidase (*AOX1*) promoter controls expression of alcohol oxidase, which catalyzes the first step in methanol metabolism. Typically, 30% of the total soluble protein in methanol-induced cells is alcohol oxidase.[1] Several *Pichia* expression vectors take advantage of the powerful *AOX1* promoter and use methanol to induce high-level expression of your gene of interest. If you prefer expression that is not dependent on methanol induction, constitutive expression can be achieved using the promoter from the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene.[2] Both inducible and constitutive expression constructs integrate into the *P. pastoris* genome, creating a stable host that generates extremely high protein expression levels.

S. cerevisiae *P. pastoris*

Figure 1—High biomass of *Pichia pastoris*.

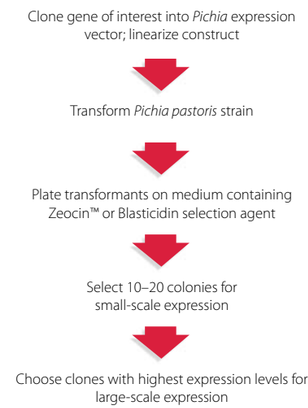


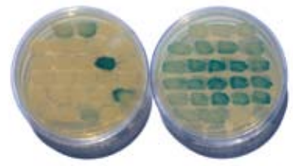
Figure 2—Overview of expression in *Pichia*.

Easy-to-use system

The *Pichia* Expression System has been engineered to make it as easy to use as bacterial systems (Figure 2). It offers the following advantages:

- → A simple, convenient method for rapidly preparing and transforming competent *P. pastoris* cells, eliminating the tedious and time-consuming preparation of spheroplasts
- → Vectors containing the Zeocin™ or blasticidin resistance gene to allow direct selection of transformed cells (Figure 3); no more hassling with drop-out medium or screening through background colonies
- → A growth medium that does not require growth factors or supplements, which are costly and make protein purification more difficult

In addition, vectors have been designed with fusion tags that simplify the purification and analysis of expressed proteins.



Histidine selection Zeocin™ selection

Figure 3—Improved selection with Zeocin™ selection agent. The *lacZ* gene was cloned into a *HIS4*-based *Pichia* vector and the Zeocin™-resistant pPICZ B *Pichia* vector. DNA was linearized at a unique restriction site in the *AOX1* region. GS115 *P. pastoris* cells were transformed by electroporation with the linearized constructs. Transformants were plated on the appropriate medium (RDB minus histidine or YPDS plus 100 µg/ml Zeocin™ agent). Colonies were selected from each plate and patched onto YPM (yeast, peptone, methanol) plates containing X-gal. Untransformed GS115 was used as a control (bottom right patch). One hundred percent of the pPICZ B–transformed colonies were blue, indicating that they contain the *lacZ* gene. By contrast, only 19% of the colonies selected by histidine prototrophy were blue.

Scale it up

Because *Pichia pastoris* is easily adaptable to large-scale fermentation, the *Pichia* Expression System is ideal for large-scale production of functional protein. *Pichia pastoris* has been used to produce recombinant protein in fermentors as large as 10,000 L. This level of scalability means more efficient protein production and, ultimately, significant cost savings.

Proven expression

Many proteins have been expressed in the *Pichia* Expression System, including enzymes, proteases, protease inhibitors, receptors, single-chain antibodies, and regulatory proteins (Table 1). Some have been expressed to levels as high as grams per liter. This proven track record means you can rely on the *Pichia* Expression System for the expression of your protein.

Table 1—Examples of heterologous proteins expressed in *Pichia pastoris*.

Protein expressed	Expression level (mg/L)	Reference
Bacterial proteins		
Tetanus toxin fragment C	12,000	Clare, J.J. et al. (1991) *Bio/Technology* 9: 455–460
α-amylase	2,500	Paifer, E. et al. (1994) *Yeast* 10: 1415–1419
T2A peroxidase	2,470	Thomas, L. et al. (1998) *Can J Microbiol* 44: 364–372
C. *botulinum* neurotoxin fragment	78	Smith, L.A. (1998) *Toxicon* 36: 1539–1548
Yeast proteins		
Catalase L	2,300	Calera, J.A. et al. (1997) *Infect Immun* 65: 4718–4724
Glucoamylase	400	Fierobe, H.-P. et al. (1997) *Protein Expr Purif* 9: 159–170
Lipase	60	Minning, S. et al. (1998) *J Biotechnol* 66: 147–156
Plant proteins		
Hydroxynitrile lyase	22,000	Hasslacher, M. et al. (1997) *Protein Exp Purif* 11: 61–71
Wheat lipid transfer protein	720	Klein, C. et al. (1998) *Protein Expr Purif* 13: 73–82
Aeroallergen	60	Huecas, S. et al. (1999) *Eur J Biochem* 261: 539–546
Invertebrate proteins		
Hirudin	1,500	Rosenfeld, S.A. et al. (1996) *Protein Expr Purif* 8: 476–482
Spider dragline silk protein	663	Fahnestock, S.R. et al. (1997) *Appl Micro Biotechnol* 47: 33–39
Honeybee olfactory protein	200	Danty, E. et al. (1999) *J Neuroscience* 19: 7468–7475
Mammalian proteins		
Mouse gelatin	14,800	Werten, M.W. et al. (1999) *Yeast* 15: 1087–1096
Porcine carboxypeptidase B	200	Ventura, S. et al. (1999) *J Biol Chem* 274: 19925–19933
Human tumor necrosis factor	10,000	Sreekrishna, K. et al. (1989) *Biochemistry* 28: 4117–4125
Human IGF-1	600	Brierley, R.A. (1998) *Methods Mol Biol* 103: 149–177
Human CD38	455	Munshi, C.B. (1997) *Methods Enzymol* 280: 318–330
15N-interferon τ	10	Johnson, T.M. et al. (1999) *J Interferon Cytokine Res* 19: 631–636

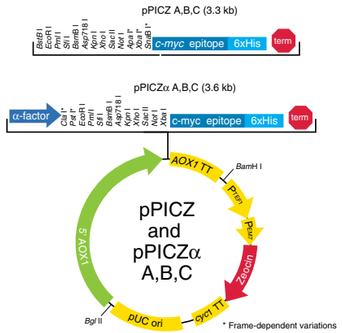
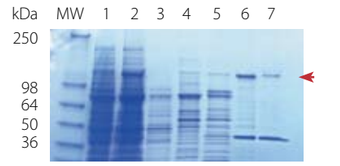


Figure 4—Vector map of pPICZ/pPICZα.



Lane 1: GS115 (cell lysate)
Lane 2: pPICZ/β-gal/GS115 (cell lysate)
Lane 3: Flow-through
Lanes 4–7: 50, 200, 350, and 500 mM imidazole elutions

Figure 5—Expression of β-galactosidase from pPICZ. The gene encoding β-galactosidase was cloned into pPICZ B in frame with the C-terminal tag. The construct was used to transform GS115 *P. pastoris* cells. Zeocin™ resistant clones were selected and one chosen for expression. Cells were grown in BMGY medium to OD_{600} = 8.0, then concentrated into 5 ml of BMMY and induced with methanol for 4 days. Cells were harvested and lysed in 500 µl of breaking buffer. Cell lysate was diluted with 3.5 ml of native binding buffer (20 mM $NaPO_4$, 500 mM NaCl, pH 7.8) and loaded onto a 2 ml ProBond™ column. Protein was eluted with increasing concentrations of imidazole. Samples were analyzed on a 4–20% Coomassie® blue–stained SDS-PAGE gradient gel. Arrow indicates the β-galactosidase protein.

Powerful, versatile tools

Pichia expression vectors offer you a variety of features that give you the ability to design an expression experiment that best meets your needs. These powerful, versatile tools are available with your choice of an inducible or constitutive promoter, with a Zeocin™ or blasticidin resistance selectable marker, and with or without a secretion signal.

Inducible expression with Zeocin™ selection

The pPICZ and pPICZα vectors (Figure 4) allow tightly regulated high-level expression. Both vectors carry the Zeocin™ resistance marker, which allows direct selection of recombinants and multi-copy integrants without the use of drop-out medium. In addition, Zeocin™ selection can be used in *E. coli*, eliminating the need for a second antibiotic marker and dramatically reducing vector size. pPICZ and pPICZα vectors also offer the following features:

- → *AOX1* promoter for high-level, methanol-induced expression
- → C-terminal *c-myc* epitope and polyhistidine (6xHis) sequence for efficient detection and rapid purification of recombinant proteins (Figure 5)
- → 5′ *AOX1* gene for targeted integration into the *Pichia* host genome

The pPICZα vector also contains the α-factor secretion signal to target recombinant proteins to the growth medium.

Constitutive expression with Zeocin™ selection

The pGAPZ and pGAPZα vectors allow you to express your protein without the use of methanol. This is sometimes preferable when performing large-scale expression. The pGAPZ and pGAPZα vectors (Figure 6) offer:

- → *GAP* promoter for high-level, constitutive expression
- → Zeocin™ resistance gene for direct selection of recombinants
- → C-terminal *c-myc* epitope and polyhistidine (6xHis) sequence for efficient detection and rapid purification of recombinant proteins

The pGAPZα vector also contains the α-factor secretion signal to target recombinant proteins to the growth medium.

Inducible expression with blasticidin selection

As an alternative to Zeocin™ selection, the pPIC vectors are available with the blasticidin resistance gene. The pPIC6 and pPIC6α vectors (Figure 7) offer the following:

- → *AOX1* promoter for high-level, methanol-induced expression
- → Blasticidin resistance gene for direct selection of recombinants
- → C-terminal *c-myc* epitope and polyhistidine (6xHis) sequence for efficient detection and rapid purification of recombinant proteins
- → 5′ *AOX1* gene for targeted integration into the *Pichia* host genome

The pPIC6α vector also contains the α-factor secretion signal to target recombinant proteins to the growth medium.

Multi-copy integration

Several *Pichia* expression vectors are available that allow you to increase the number of copies of your gene of interest in *P. pastoris*. This may lead to higher expression of your protein. The pPIC3.5K and pPIC9K vectors carry the kanamycin resistance gene to allow selection of transformants in which multiple copies of a *Pichia* expression vector have

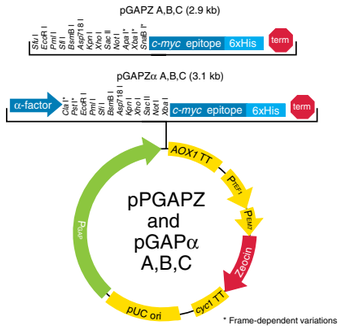


Figure 6—Vector map of pGAPZ/pGAPZα.

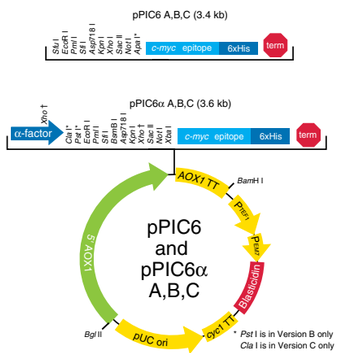


Figure 7—Vector map of pPIC6/pPIC6α.

spontaneously integrated. Spontaneous multiple insertion events can be identified by their increased Geneticin® resistance. The pAO815 vector provides an alternative to screening for multiple insertion events. This vector allows you to clone multiple copies of your gene into a single vector. With pAO815, you can control the number of copies of your gene you want to express. The pPIC3.5K, pPIC9K, and pAO815 vectors (Figure 8) all carry the following features:

- → *AOX1* promoter for high-level inducible expression
- → *HIS4* gene for identification of transformants
- → 3′ *AOX1* gene for targeted integration into the *Pichia* host genome

In addition, the pPIC9K vector carries the α-factor secretion signal to target recombinant proteins to the growth medium. For more information about the pPIC3.5K, pPIC9K, and pAO815 vectors, visit www.invitrogen.com.

Optimize with *Pichia* strains

Selection and expression in *P. pastoris* are made easier with our large collection of strains. These strains allow you to optimize expression and recovery of your protein of interest. Table 2 provides information to help you choose the appropriate strain.

Table 2—*Pichia pastoris* strains.

Strain	Genotype	Application
GS115	*his4*	Selection of expression vectors containing *HIS4*
X-33	Wild type	Selection of Zeocin™-resistant expression vectors
KM71	*his4, aox1::ARG4, arg4*	Selection of expression vectors containing *HIS4* to generate strains with Mut^S phenotype
KM71H	*aox1::ARG4, arg4*	Selection of Zeocin™-resistant expression vectors to generate strains with Mut^S phenotype
SMD1168	*his4, pep4*	Selection of expression vectors containing *HIS4* to generate strains without protease A activity
SMD1168H	*pep4*	Selection of Zeocin™-resistant expression vectors to generate strains without protease A activity

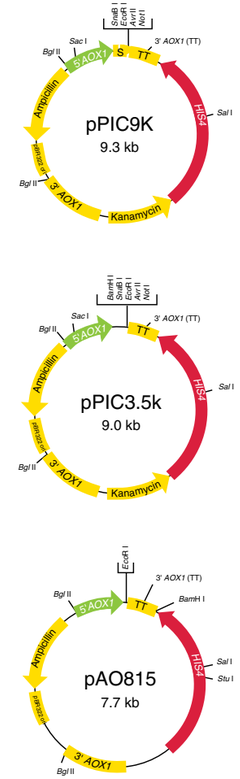


Figure 8—The multi-copy *Pichia* vectors.