2 Bacterial Strains for Protein Expression

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Protein expression in *Escherichia coli (E. coli)* has been a popular means of producing recombinant proteins for several decades. *E. coli* is a well-established host that offers easy genetic manipulation, short and inexpensive culture. Additionally, *E. coli* has a long history of being able to produce many different types of proteins.

The T7 RNA Polymerase System is the most popular approach for producing proteins in *E. coli*. In this system, an expression vector containing a gene of interest, cloned downstream of the T7 promoter, is introduced into a T7 expression host. T7 expression hosts such as DE3 strains have a chromosomal copy of the phage T7 RNA polymerase gene. When an inducer such as IPTG or rhamnose is added to the culture, T7 RNA polymerase is expressed and transcribes the gene of interest, followed by translation of the desired protein by endogenous protein translation machinery.

Promega offers ready–to–use competent cells for expression of recombinant proteins in *E. coli*.

Single-Step (KRX) Competent Cells

Tightly-controlled protein expression in *E. coli* based on T7 RNA polymerase rhamnose-inducible system.

Description

The Single-Step (KRX) Competent Cells are an *E. coli* K strain that is designed for both efficient transformation (>10^8 cfu/µg) and tightly-controlled protein expression. The stringent control provided by the rhamnose-driven T7 RNA polymerase may allow cloning of proteins toxic to *E. coli.* The KRX Single-Step Competent Cells are available in convenient single transformation size (50µl aliquots).

Principle

Single-Step (KRX) Competent Cells contain a chromosomal copy of the T7 RNA polymerase driven by a rhamnose promoter (rhaBAD) that provides tight control of the proteins expressed via a T7 promoter **(Figure 2.1)**. Addition of rhamnose induces the expression of the T7 RNA polymerase, which in turn transcribes the gene of interest under control of a T7 promoter. Protein expression level in KRX cells are as high or higher than levels expressed in BL21(DE3)-derived strains. However, pre-induction protein expression levels in Single-Step (KRX) Competent Cells are significantly lower than those of BL21(DE3)-derived strains **(Figure 2.2)** and therefore recommended for the expression of protein toxic to *E. coli*.

Features and Benefits

- **Save Time:** Clone and express your vector in one step.
- **Controlled Protein Expression:** Highly regulatable protein expression.
- **Achieve High Yields:** Protein expression as high or higher than levels expressed in BL21(DE3)-derived strains.
- **Blue/White Screening:** Convenient method for detecting recombinant clones.

Additional Information:

KRX Genotype: [F′, *tra*D36, Δ*omp*P, *pro*A$^+$B$^+$, lacI^q, Δ(*lacZ*) M15] Δ*omp*T, *end*A1, *rec*A1, *gyr*A96 (Nal^r), *thi*-1, *hsd*R17 (r_k^-, m_k^+), e14$^-$ (McrA$^-$), *rel*A1, *sup*E44, Δ(*lac-pro*AB), Δ(*rha*BAD)::T7 RNA polymerase.

Single-Step (KRX) Competent Cells

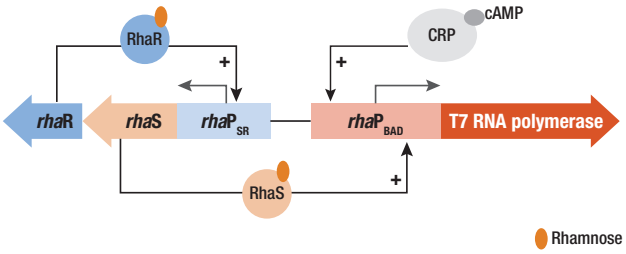

Figure 2.1. Tightly controlled inducible expression with L-Rhamnose in KRX *E. coli*. T7 RNA polymerase expression is under the control of the $rhaP_{BAD}$ promoter in the KRX strain. This promoter is subject to multiple levels of control. In the presence of preferred carbon sources, such as glucose, cyclic AMP (cAMP) concentrations are low and the cAMP receptor protein (CRP) does not activate transcription. Upon depletion of glucose, cAMP levels rise and CRP can activate transcription at $rhaP_{BAD}$. In addition, L-rhamnose can bind to RhaR, which binds the rhaPSR promoter, resulting in the production of active RhaS and more RhaR. RhaS also binds rhamnose, which then binds the $rhaP_{BAD}$ promoter, resulting in the production of high levels of T7 RNA polymerase. The T7 RNA polymerase in turn transcribes the gene of interest.

References

Malu, B *et al.* (2013) A nondiscriminating glutamyl-tRNA synthetase in the plasmodium apicoplast: the first enzyme in an indirect aminoacylation pathway. *J. Biol. Chem.* **288**(45), 32539–52.

Barquilla, A *et al.* (2012) Third target of rapamycin complex negatively regulates development of quiescence in Trypanosoma brucei. *Proc.Natl. Acad. Sci*, **109**(36), 14399–404.

Ordering Information

Single-Step (KRX) Competent Cells (Cat.# L3002)

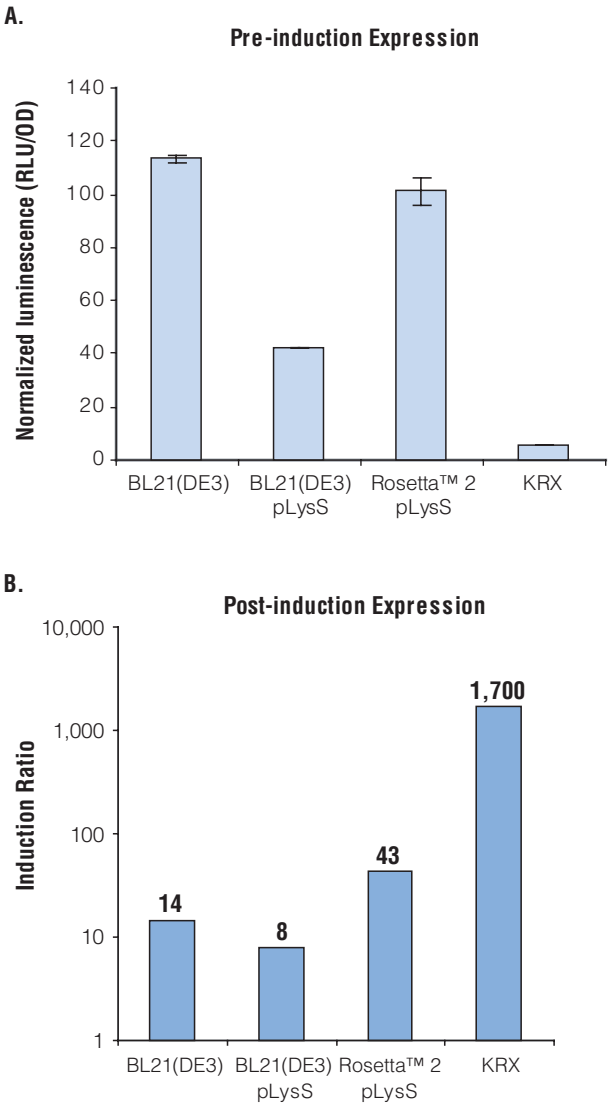

Figure 2.2. Pre-induction and post-induction expression levels of firefly luciferase. KRX shows only very low pre-induction **(Panel A)** and very high post-induction levels **(Panel B)** compared to other strains such as BL21(DE3).

BL21 Competent Protein Expression Cells

Inducible recombinant protein expression *in E. coli.*

Description

BL21(DE3)pLysS Competent Cells and Single-Use BL21(DE3)pLysS Competent Cells allow high-efficiency protein expression of any gene that is under the control of a T7 promoter. The strain carries both the DE3 lysogen and the plasmid pLysS. pLysS constitutively expresses low levels of T7 lysozyme, which reduce basal expression of recombinant genes by inhibiting basal levels of T7 RNA polymerase. High protein expression is achieved by IPTG addition. Competent cells are available in standard format (200µl aliquots) as well as in 50µl aliquots.

Principle

BL21(DE3)pLysS is a derivative of BL21 that has the T7 RNA polymerase gene under the control of the lacUV5 promoter. This arrangement is on a phage genome, called DE3. DE3 is inserted into the chromosome of BL21 to make BL21(DE3). pLysS is a plasmid that contains the T7 lysozyme gene (LysS). The T7 lysozyme binds to T7 RNA polymerase causing inhibition until induction by the addition of IPTG. When IPTG is added, the amount of T7 RNA polymerase increases and overcomes the inhibition by LysS.

Features and Benefits

- **T7 RNA Polymerase under the Control of the lac UV5 Promoter:** Inducible protein expression.
- **Deficient in Proteases lon and OmpT:** Increased stability of expressed protein.
- **pLysS Plasmid:** Lower background expression of target genes.

Additional Information:

Genotype: F–, *ompT*, *hsdSB* (r_B–, m_B–), *dcm*, *gal*, λ(DE3), pLysS, Cm^r.

References

Firdaus, M. *et al.* (2013) The pH sensitivity of murine heat shock protein 47 (HSP47) binding to collagen is affected by mutations in the breach histidine cluster. *J. Biol. Chem.* **288**(6), 4452–61

Otsu, W. *et al.* (2013) A new class of endoplasmic reticulum export signal PhiXPhiXPhi for transmembrane proteins and its selective interaction with Sec24C. *J. Biol. Chem*. **288**(25), 18521–61

Yamazaki, D. *et al.* (2013) srGAP1 regulates lamellipodial dynamics and cell migratory behavior by modulating Rac1 activity. *Mol.Biol. Cell.* **24**(21), 3393–05

Ordering Information

BL21(DE3)pLysS Competent Cells (Cat.# L1191)

Single-Use BL21(DE3)pLysS Competent Cells (Cat.# L1195)