TECHNICAL BULLETIN

Single Step (KRX) Competent Cells

Instructions for Use of Product
L3002

Revised 5/20
TB352

Single Step (KRX) Competent Cells

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1. Description

Single Step (KRX) Competent Cells[a] are prepared from the KRX strain according to a modified procedure of Hanahan (1). KRX is an *E. coli* K12 derivative that has salient features associated with cloning and screening strains plus engineered attributes to optimize controlled protein expression. Attributes that make this a good cloning strain are the partially defective restriction systems (*hsd* and e14), a lack of the most common nuclease that copurifies with plasmid DNA isolated from *E. coli* strains (*end*A$^-$) and a preventive mutation to minimize undesirable recombination events (*rec*A$^-$). KRX can be used for blue/white screening because it is deficient in β-galactosidase activity due to deletions in both genomic and episomal copies of the *lac*Z gene. The partial deletion in the episomal (F´ factor) copy of the *lac*Z gene (Δ(*lac*Z)M15) can be complemented by adding a functional α-peptide encoded by a plasmid cloning vector.

KRX also has attributes that make it a good protein expression strain. The *omp*T$^-$ and *omp*P$^-$ mutations eliminate one source of proteolysis of overexpressed protein in *E. coli*.

KRX incorporates a chromosomal copy of the T7 RNA polymerase gene driven by the rhamnose promoter (*rha*P$_{BAD}$) to provide dramatic control of recombinant protein expression. T7 RNA polymerase-based systems (2) are among the most widely used protein expression systems by virtue of the well-defined promoter, which is completely independent of *E. coli* RNA polymerase promoters, and the rapid elongation rate exhibited by T7 polymerase, which is about five times that of *E. coli* RNA polymerase (3). Since this system has been in use for many years, many vectors are commercially available to overproduce proteins in *E. coli* using the T7 promoter, including the Promega Flexi® Vectors.

1. Description (continued)

The T7 RNA polymerase gene replaces the *rha*BAD genes such that its expression is controlled by the *rha*BAD promoter (*rha*P_{BAD}). This promoter is subject to catabolite repression by glucose and activated by adding rhamnose to the medium, thus providing precise control of T7 RNA polymerase abundance and thereby precise control of recombinant protein production. *rha*P_{BAD}-driven expression is positively controlled through a regulatory cascade of two activators. Rhamnose induces the activator RhaR, which induces production of active RhaS, which in turn binds rhamose, activating transcription from *rha*P_{BAD} (4,5, Figure 1). Since the isomerase (RhaA), kinase (RhaB) and aldolase (RhaD) are deleted and replaced with the gene for T7 RNA polymerase in KRX, rhamnose is not metabolized by the cell and is not consumed during growth.

KRX Genotype

[F´, *tra*D36, Δ*omp*P, *pro*A^+B^+, *lac*I^q, Δ(*lac*Z)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

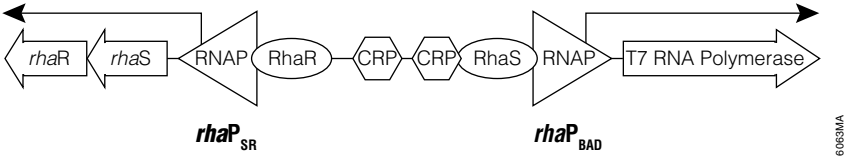

Figure 1. Rhamnose control of T7 RNA polymerase in KRX. T7 RNA polymerase is expressed by the *rha*P_{BAD} promoter in the KRX strain. This promoter is subject to multiple levels of positive control (4). In the presence of preferred carbon sources, such as glucose, cyclic AMP (cAMP) concentrations are low and the cAMP receptor protein (CRP) does not activate transcription. Upon depletion of glucose, cAMP levels rise and CRP can activate transcription at *rha*P_{BAD}. In addition, L-rhamnose can bind to RhaR, which binds the *rha*P_{SR} promoter, resulting in the production of active RhaS and more RhaR. RhaS also binds rhamnose, which then binds the *rha*P_{BAD} promoter, resulting in the production of high levels of T7 RNA polymerase.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Single Step (KRX) Competent Cells (>10^8 cfu/µg)	**20 × 50µl**	**L3002**

Storage Conditions: Always store competent cells at –70°C. Thaw on ice when ready for use. Do not refreeze thawed, unused aliquots.

Cells are supplied in 50µl aliquots. Typically, 50–100µl of competent cells is required for a standard transformation.

3. Standard Transformation Protocol

Materials to Be Supplied by the User

(Solution compositions are provided in Section 6.)

- LB or SOC medium
- LB plates with antibiotic appropriate for the plasmid
- IPTG (Cat.# V3955; optional, see Note 4)
- X-Gal (Cat.# V3941; optional, see Note 4)

1. Remove Single Step (KRX) Competent Cells from –70°C and place on ice for 5 minutes or until just thawed.
2. Add 1–50ng of DNA (in a volume not greater than 5µl) to the Single Step (KRX) Competent Cells. Move the pipette tip through the cells while dispensing. Quickly flick the tube several times. **Do not vortex!**

   Note: To determine transformation efficiency, we recommend using 2µl of supercoiled plasmid DNA (e.g., pGEM®-3Z Vector, Cat.# P2151) diluted to 5pg/µl in TE buffer. See Section 5 for more information.
3. Immediately return the tubes to ice for 5–30 minutes (Figure 2).
4. Heat-shock cells for 15–20 seconds in a water bath at exactly 42°C (Figure 3). Do not shake.
5. Immediately place the tubes on ice for 2 minutes.
6. Add 450µl of room-temperature SOC medium to each transformation reaction, and incubate for 60 minutes at 37°C with shaking (approximately 225rpm). For best transformation efficiency, lay the tubes on their sides and tape them to the platform.
7. For each transformation reaction, we recommend plating 100µl of undiluted cells and 1:10 and 1:100 cell dilutions on antibiotic plates (see Notes 1–4). Incubate the plates at 37°C overnight.

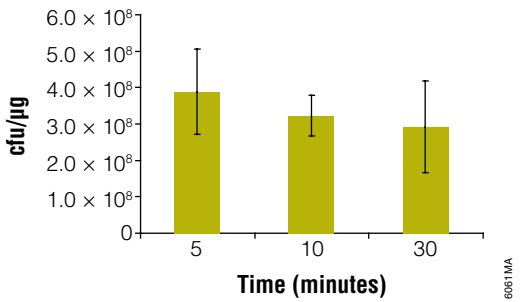

Figure 2. Effect of ice incubation duration on transformation efficiency. Single Step (KRX) Competent Cells were transformed according to the protocol described in Section 3. Cells were incubated on ice for 5, 10 or 30 minutes in Step 3 before they were heat shocked at 42°C for 15 seconds.

3. Standard Transformation Protocol (continued)

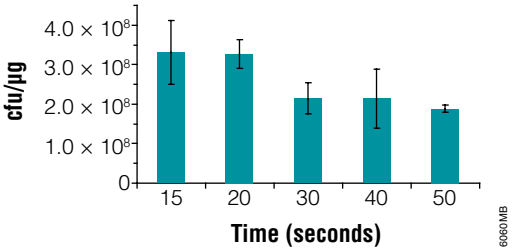

Figure 3. Effect of heat shock duration on transformation efficiency. Single Step (KRX) Competent Cells were transformed according to the protocol described in Section 3. Cells were incubated on ice for 30 minutes in Step 3 before they were heat shocked at 42ºC for various times.

Notes:

1. For control transformations using supercoiled plasmid DNA (e.g., pGEM®-3Z Vector, Cat.# P2151), we recommend diluting transformed cells 1:4 before plating 100µl on LB/ampicillin plates.
2. If more colonies are desired, pellet the cells by centrifugation at 1,000 × *g* for 10 minutes and resuspend in 200µl of SOC or LB medium and plate.
3. Use high-quality deionized water (e.g., Milli-Q® or NANOpure® water) for SOC medium (see Section 6). If LB or other medium is used, transformation efficiency will be reduced.
4. Blue/white screening can be used with a variety of vectors in conjunction with Single Step (KRX) Competent Cells. To use blue/white color screening for identifying recombinants, plate transformed cells on LB plates containing 100µg/ml ampicillin, 0.5mM IPTG (Cat.# V3955) and 40µg/ml X-Gal (Cat.# V3941). Incubate overnight at 37°C.

   An alternative to preparing plates containing X-Gal and IPTG is to spread 20µl of 50mg/ml X-Gal and 100µl of 0.1M IPTG onto LB ampicillin plates and allow these components to absorb for 30 minutes at 37°C prior to plating cells.
5. Solutions and media containing tetracycline must be stored protected from light to maintain potency.
6. If plasmid stability is suspect, for example, when expressing proteins that may be toxic to *E. coli*, add glucose (0.4% final concentration) to the LB plates. This will reduce basal expression of T7 RNA polymerase in KRX and thereby reduce expression of toxic proteins.

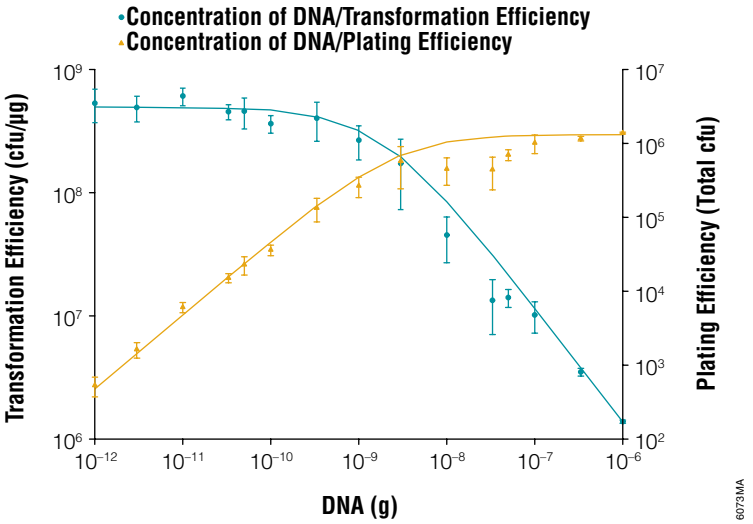

Figure 4. Effect of DNA concentration on transformation and plating efficiency. Single Step (KRX) Competent Cells were transformed as described in Section 3. The amount of DNA varied from 1pg to 1µg.

4. Guidelines for Protein Expression in KRX Strains

These guidelines are for protein-coding regions under the control of a T7 promoter, such as in pET Vectors (Novagen) or Flexi® Vectors (e.g., pF1A, pF1K, pFN2A, pFN2K, pFN6A, pFN6K, pFC7A and pFC7K).

1. Grow starter cultures overnight in LB medium containing antibiotic appropriate for the plasmid at 37°C with shaking at approximately 275rpm. These cultures can be supplemented with 0.4% glucose (1:50 dilution of 20% glucose) to further reduce background expression of the protein, which could be useful when expressing proteins normally toxic to *E. coli*.
2. Dilute overnight starter cultures 1:100 into LB or Terrific Broth containing antibiotic appropriate for the plasmid. Terrific Broth is preferred since it results in greater final cell mass. Grow cultures at 37°C with shaking at approximately 275rpm until they reach an optical density ($O.D._{600}$) of 0.4–0.5 for LB cultures or 0.8–1.0 for Terrific Broth cultures.
3. Shift cultures to another incubator shaker set at 15–25°C, and continue shaking at approximately 275rpm.
4. When the cultures reach an $O.D._{600}$ of 0.5–0.6 for LB or 1.0–1.5 for Terrific Broth, induce protein expression by adding rhamnose to a concentration of 0.1% (1:200 dilution of 20% rhamnose). Lower levels of rhamnose will result in lower levels of induction, which may assist production of soluble expressed protein. You may need to adjust the rhamnose concentration to balance expression yield and solubility. Many pET vectors, pF1A and pF1K also contain *lac* operators adjacent to or overlapping the T7 promoter. Maximal expression from these plasmids may require adding IPTG (1mM final concentration).

4. Guidelines for Protein Expression in KRX Strains (continued)

5. Grow cultures overnight at 15–25°C with shaking at approximately 275rpm.
6. Harvest cells by centrifugation (e.g., 10,000 × *g* for 3 minutes in a microcentrifuge or 4,000 × *g* for 10 minutes in a larger format).
7. Cells can be lysed by mechanical means (e.g., sonication, French Press or BeadBeater-type homogenizers) or by chemical means (FastBreak™ Cell Lysis Reagent, Cat.# V8571).

   Note: When analyzing expression by SDS-PAGE, a 98,885Da molecular weight band (T7 RNA polymerase) may appear after induction.

5. Calculation of Transformation Efficiency (Colony Forming Units [cfu])

Transformation efficiency is defined as the number of colony forming units (cfu) produced by 1µg of supercoiled plasmid DNA (e.g., pGEM®-3Z Vector, Cat.# P2151) and is measured by performing a control transformation reaction using a known quantity of DNA, typically 0.1ng and calculating the number of cfu formed per microgram DNA.

Equation for Transformation Efficiency (cfu/µg)

$$\frac{\text{number of cfu on control plate}}{\text{ng of supercoiled DNA plated}} \times \frac{1 \times 10^3\,\text{ng}}{\mu\text{g}}$$

Example:

A total of 900µl of SOC medium is added to 100µl of competent cells that have been transformed with 0.1ng supercoiled plasmid. 100µl (equivalent to 0.01ng) of this dilution is transferred to 900µl of SOC medium, and 100µl (equivalent to 0.001ng) is plated. If 100 colonies are observed on the plate, the transformation efficiency is:

$$\frac{100\text{cfu}}{0.001\text{ng}} \times \frac{1 \times 10^3\,\text{ng}}{\mu\text{g}} = 1 \times 10^8\,\text{cfu}/\mu\text{g}$$

Note: Transformation with plasmid DNA in ligation mixtures will produce fewer colonies than transformation with supercoiled plasmid DNA.

6. Composition of Buffers and Solutions

glucose, 2M

180.16g glucose

Add distilled water to 500ml, sterilize through a 0.2µm filter unit and store in aliquots at –20°C.

glucose, 20% (w/v)

20g D-glucose

Add distilled water to 100ml, sterilize through a 0.2µm filter unit and store in aliquots at –20°C.

IPTG stock solution, 0.1M

1.2g IPTG (Cat.# V3955)

Add water to 50ml final volume. Sterilize through a 0.2µm filter unit, and store at 4°C.

LB medium with or without antibiotic

10g/L Bacto®-tryptone
5g/L Bacto®-yeast extract
5g/L NaCl

Adjust the pH to 7.5 with NaOH. Autoclave to sterilize. Allow the autoclaved medium to cool to 55°C, and add antibiotic to a final concentration as shown in Table 1. For LB plates, include 15g agar prior to autoclaving.

Mg^{2+} stock solution, 2M

101.5g $MgCl_2 \cdot 6H_2O$
123.3g $MgSO_4 \cdot 7H_2O$

Add distilled water to 500ml, and sterilize through a 0.2µm filter unit.

potassium phosphate, 0.89M

23.1g KH_2PO_4 (monobasic)
125.4g K_2HPO_4 (dibasic)

Add distilled water to 1,000ml, and sterilize through a 0.2µm filter unit.

rhamnose, 20% (w/v)

10g L-rhamnose monohydrate (Cat.# L5701)

Add distilled water to 45ml, sterilize through a 0.2µm filter unit and store in aliquots at –20°C.

6. Composition of Buffers and Solutions (continued)

SOC medium

2.0g	Bacto®-tryptone
0.5g	Bacto®-yeast extract
1ml	1M NaCl
0.25ml	1M KCl
1ml	Mg^{2+} stock solution, 2M
1ml	2M glucose, filter-sterilized

Add Bacto®-tryptone, Bacto®-yeast extract, NaCl and KCl to 97ml distilled water. Stir to dissolve. Autoclave, and cool to room temperature. Add sterile 2M Mg^{2+} stock and 2M glucose stock, each to a final concentration 20mM. Bring to 100ml with distilled water. Filter through a sterile 0.2µm filter unit.

Terrific Broth

12.0g	Bacto®-tryptone
24.0g	Bacto®-yeast extract
4ml	glycerol
100ml	potassium phosphate, 0.89M

Add Bacto®-tryptone, Bacto®-yeast extract, glycerol to 750ml distilled water. Stir to dissolve, then bring the volume to 900ml with distilled water. Autoclave, and cool to 60ºC. Add 100ml of sterile 0.89M potassium phosphate to a final volume of 1,000ml. Filter through a sterile 0.2µm unit.

X-Gal

Available from Promega (Cat.# V3941) at a concentration of 50mg/ml in dimethylformamide.

Note for all filter sterilized solutions: Filter-sterilizing units should be prerinsed with distilled water before use to remove any toxic material.

Table 1. Antibiotics Useful for Plasmid Selection in KRX.

Antibiotic	Plasmid Marker	Final Concentration
Ampicillin	*bla*, Amp^r	100µg/ml
Carbenicillin	*bla*, Amp^r	50µg/ml
Kanamycin	Kan^r	30µg/ml
Tetracycline	Tet^r	12.5µg/ml
Chloramphenicol	Cm^r, Cam^r	34µg/ml

7. References

1. Hanahan, D. (1985) In: *DNA Cloning*, Vol. 1, Glover, D., ed., IRL Press, Ltd., 109.
2. Studier, F.W. and Moffat, B.A. (1986) Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189**, 113–30.
3. Golomb, M. and Chamberlin, M. (1974) Characterization of T7-specific ribonucleic acid polymerase. IV. Resolution of the major in vitro transcripts by gel electrophoresis. *J. Biol. Chem.* **249**, 2858–63.
4. Egan, S.M. and Schleif, R.F. (1993) A regulatory cascade in the induction of *rha*BAD. *J. Mol. Biol.* **234**, 87–98.
5. Haldimann, A., Daniels, L. and Wanner, B. (1998) Use of new methods for construction of tightly regulated arabinose and rhamnose promoter fusions in studies of the *Escherichia coli* phosphate regulon. *J. Bacteriol.* **180**, 1277–86.

8. Related Products

Product	Size	Cat.#
L-Rhamnose Monohydrate	10g	L5701
	50g	L5702
pGEM®-3Z Vector	20µg	P2151
X-Gal	100mg	V3941
IPTG, Dioxane Free	1g	V3955
pF1A Flexi® Vector	20µg	C8441
pF1K Flexi® Vector	20µg	C8451
pFN2A Flexi® Vector	20µg	C8461
pFN2K Flexi® Vector	20µg	C8471

9. Summary of Changes

The following changes were made to the 5/20 revision of this document:

1. Updated Section 8, Related Products to remove discontinued products and correct the pFN2K Flexi® Vector catalog number.

[a]Patent Pending.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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