Protein Expression in Less Time: A Short Induction Protocol for KRX

ABSTRACT Single Step (KRX) Competent Cells are ideal for rapidly generating new clones for recombinant protein expression. We examined the effect of induction time and media on recombinant protein yield and activity in KRX. Firefly and *Renilla* luciferases were used as models. Induction times as brief as 4 hours were sufficient to produce significant quantities of active protein. Using Single Step (KRX) Competent Cells with this short induction protocol, protein can be expressed and analyzed in less time, without the need for overnight induction.

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Day Zero
Transform Single Step (KRX) Competent Cells and plate with selection.

Day One
Pick colonies and inoculate cultures.

Day Two
Dilute cultures 1:100, grow to ideal density, reduce temperature and induce.

Harvest
after 4 hours.

INTRODUCTION

KRX bacteria are a strain of *E. coli* engineered for cloning, screening and expressing proteins (1). The Single Step (KRX) Competent Cells[a] (Cat.# L3001) are highly competent, allowing efficient transformation of ligation reactions, and they allow blue/white screening. They also have mutations to maintain plasmid quality and insert stability (e.g., *end*A–, *rec*A–).

For protein expression, the KRX bacteria has a chromosomal copy of the T7 RNA polymerase gene driven by a rhamnose promoter. The rhamnose promoter is repressed by glucose and highly activated by rhamnose, giving precise control of recombinant protein expression. KRX also contains mutations to minimize proteolysis of over-expressed proteins (*omp*T– and *omp*P–), thus improving protein yield and integrity.

For maximum protein expression, inductions are traditionally done overnight. While this can result in higher protein yields, long inductions can have a negative impact on protein activity (2). Here we examined protein expression and activity at various times after induction in standard Luria broth (LB) and compared activity to rich Terrific Broth (TB).

EFFECT OF TIME ON PROTEIN EXPRESSION LEVELS AND ACTIVITY

To examine the effect of induction time on protein expression, the pFN6A (HQ) Flexi® Vector containing the coding region of *Renilla* (pFN6A-*hRluc*) or firefly luciferase (pFN6A-*luc*) was transformed and expression induced in Single Step (KRX) Competent Cells as described in the *Single Step (KRX) Competent Cells Technical Bulletin* #TB352. Overnight cultures were grown at 37°C in the presence of 0.4% glucose to minimize background protein expression, then diluted 1:100 in 50ml of LB and incubated at 37°C until the cultures reached 0.4–0.5 $O.D._{600}$. The cultures were transferred to 25°C and induced using 0.1% rhamnose (final concentration). Aliquots were removed and measured for culture density ($O.D._{600}$) at various time points and then analyzed for luciferase activity using the *Renilla* Luciferase Assay System (Cat.# E2810) or the Bright-Glo™ Luciferase Assay System (Cat.# E2610), or lysed in FastBreak™ Cell Lysis Reagent (Cat.# V8571) for gel analysis.

An increase in *Renilla* luciferase protein was visible by gel analysis as early as 2 hours after induction (Figure 1). At 4 hours, more active *Renilla* luciferase was present per $O.D._{600}$ of culture than was present after overnight incubation. The culture density continued to increase throughout the incubation ($O.D._{600}$ = 2.61 and 5.09 for 4 hours and 20 hours, respectively); therefore, more total *Renilla* luciferase activity was measured at the later time points, although the activity per $O.D._{600}$ was lower. Firefly luciferase activity increased the most within the first 4 hours of induction, with a moderate increase continuing through later time points.

EFFECT OF MEDIA ON PROTEIN EXPRESSION AND ACTIVITY

Terrific Broth is a richer medium than LB, resulting in greater cell mass and allowing higher recombinant protein expression levels. The short 4-hour induction and standard overnight induction were compared for protein expression in LB and TB (Figure 2, Panel A). The activity of *Renilla* luciferase (Figure 2, Panel B) was significantly higher in TB versus LB, which is in direct contrast to firefly luciferase (Figure 2, Panel C). These data emphasize the need to empirically determine optimal expression conditions for each protein.

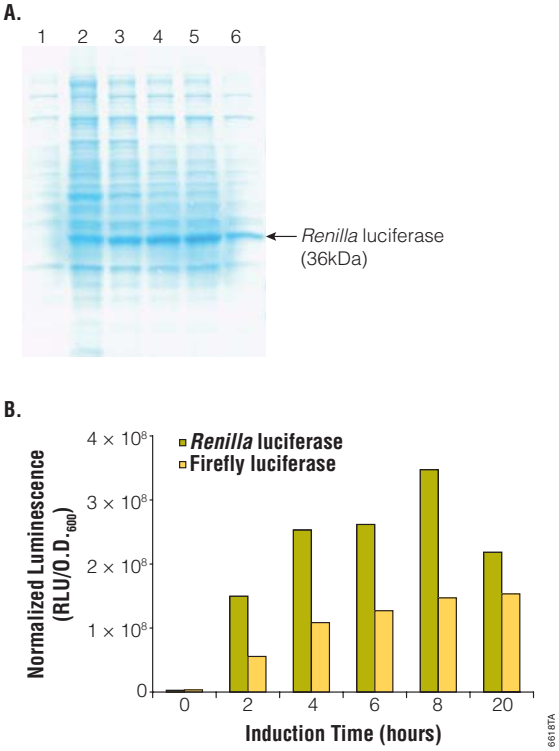

Figure 1. KRX expression of luciferases over time. KRX transformed with pFN6A-*hRluc* (*Renilla* luciferase) or pFN6A-*luc* (firefly luciferase) were induced in 50ml LB cultures at 25°C as described in the Technical Bulletin #TB352. Samples were collected before induction or at indicated time points after induction and analyzed. **Panel A.** Bacteria from 0.1 $O.D._{600}$ of pFN6A-*hRluc* cultures were lysed in FastBreak™ Reagent, run on a 4–12% Novex NuPAGE® Bis-Tris gel and visualized by SimplyBlue™ Safestain (Invitrogen). Lane 1, uninduced (0 hour); lane 2, 2-hour induction; lane 3, 4-hour induction; lane 4, 6-hour induction; lane 5, 8-hour induction; lane 6, 20-hour induction. **Panel B.** *Renilla* and firefly luciferase activities were measured by diluting cultures 1:10 in water and assaying with the *Renilla* Luciferase Assay System (20µl diluted culture + 100µl reagent) or Bright-Glo™ Luciferase Assay System (50µl diluted culture + 50µl reagent). Light output was measured on a GloMax™ 96 Microplate Luminometer (Cat.# E6501) and normalized to cell number by dividing luminescence by the $O.D._{600}$.

SUMMARY

These data demonstrate that a short 4-hour induction protocol can be used to express significant quantities of active protein from Single Step (KRX) Competent Cells. This allows induction and analysis of recombinant protein expression on the same day. Overnight induction times can be used to generate more total protein, but shorter times can give higher amounts of active protein per O.D. of culture. Optimal induction time and media conditions will depend on the protein expressed and the needs of the researcher. Additional examples of the utility of this short induction protocol are given in "Compatibility of Single Step (KRX) Competent Cells with the MagneGST™ Pull-Down System" on page 22 of this issue (3).

ACKNOWLEDGMENTS

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REFERENCES

1. Hartnett, J. *et al.* (2006) *Promega Notes* **94**, 27–30.
2. Sambrook, J. *et al.* (2001) *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
3. Schagat, T. (2007) *Promega Notes* **96**, 22–3.

ORDERING INFORMATION

Product	Size	Cat.#
Single Step (KRX) Competent Cells	5 × 200µl	L3001
	20 × 50µl	L3002

[a] Usage restrictions apply to Bacterial Strains JM109(DE3), BL21(DE3)pLysS and KRX and to any derivatives thereof. Please read the statement on page 26 describing these restrictions before purchasing any of these products.

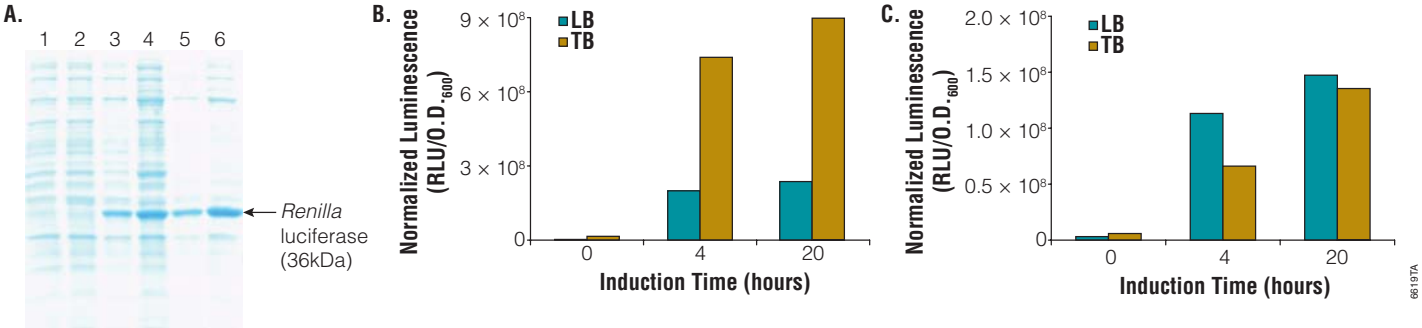

Figure 2. KRX expression of luciferases in LB versus TB. KRX transformed with pFN6A-*hRluc* (*Renilla* luciferase) or pFN6A-*luc* (firefly luciferase) were induced in 5ml LB or TB cultures at 25°C as described in the Technical Bulletin #TB352. Samples were collected before induction or at 4 or 20 hours after induction and analyzed. **Panel A.** Bacteria from 0.1 $O.D._{600}$ of pFN6A-*hRluc* cultures were lysed in FastBreak™ Reagent, run on a 4–12% Novex NuPAGE® Bis-Tris gel and visualized by SimplyBlue™ Safestain (Invitrogen). Lanes 1, 3 and 5 are samples from the LB cultures; lanes 2, 4, and 6 are samples from the TB cultures. Lanes 1 and 2, uninduced (0 hour); lanes 3 and 4, 4-hour induction; lanes 5 and 6, 20-hour induction. **Panel B.** *Renilla* luciferase activity was measured by combining 2µl of culture with 18µl of water and 100µl of *Renilla* Luciferase Assay Reagent. Light output was measured on a GloMax™ 96 Microplate Luminometer and normalized to cell number by dividing luminescence by the $O.D._{600}$. **Panel C.** Firefly luciferase activity was measured by combining 5µl of culture with 45µl of water and 50µl of Bright-Glo™ Luciferase Assay Reagent. Light output was measured and normalized as with the *Renilla* samples.