**KRX Autoinduction Protocol:**
A Convenient Method for Protein Expression

**INTRODUCTION**
Single Step (KRX) Competent Cells\(^{(a,b)}\) allow efficient transformation, cloning, screening and protein expression (1), providing researchers a single strain for all their basic molecular biology needs.

KRX is an *E. coli* K strain that contains a chromosomal copy of the T7 RNA polymerase gene tightly regulated by a rhamnose promoter (\(rhaP_{BAD}\)) (Figure 1). The \(rhaP_{BAD}\) promoter is subject to catabolite repression by glucose via cyclic AMP (cAMP) and the cAMP receptor protein (2). The presence of rhamnose activates the promoter, but only once glucose is consumed from the culture medium. This provides dramatic control of proteins expressed via a T7 promoter. Pre-induction protein expression levels are exceptionally low (1).

Proteins critical for rhamnose metabolism [isomerase (RhaA), kinase (RhaB) and aldolase (RhaD)] are deleted in KRX. Therefore rhamnose is not metabolized by the cells or consumed during growth, allowing long culture times in the presence of rhamnose.

By titrating glucose and rhamnose concentrations, we developed an autoinduction protocol that eliminates the need to monitor culture density and perform a separate induction step. We examined the effect of medium type on this process and tested the utility of the protocol by expressing three different proteins.

**GLUCOSE AND RHAMNOSE TITRATION**
To test the effect of glucose and rhamnose concentrations on protein expression in KRX, we examined the timing and amount of recombinant luciferase produced under different conditions (Figure 2). For both firefly and Renilla luciferases, there were significant differences in protein expression at 8 hours between the highest and lowest glucose concentrations tested, 0.15% and 0.05%, respectively. The greatest difference in protein expression levels for firefly luciferase was between 8 and 16 hours (10- to 100-fold, Figure 2, Panel A) and for Renilla luciferase was at 8 hours (100-fold, Figure 2, Panel B). All media yielded comparable levels of luciferase activities by 24 hours.

Rhamnose titration had a minor effect on firefly luciferase expression at high glucose concentrations. In the example shown in Figure 2, Panel A, at 16 hours high glucose and rhamnose concentrations gave low firefly luciferase activity, but the magnitude of this effect varied between experiments and was not observed for Renilla luciferase.

**ABSTRACT**
Recombinant protein expression in bacteria is a simple, yet time-consuming process. Typically cells are grown overnight to stationary phase, diluted and grown to an optimal density, then induced. To simplify this process, we took advantage of the tight regulation of protein expression by glucose and rhamnose in the KRX strain of *E. coli*. We developed an autoinduction protocol that eliminates the need for culture density monitoring and a separate induction step. We describe early and late autoinduction protocols in LB medium and demonstrate autoinduced expression of three different proteins.

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EFFECT OF MEDIA ON AUTOINDUCTION

Luria Bertani medium (LB) is most commonly used for bacterial culture and protein expression. Rich media such as Terrific Broth (TB) can be used to support higher density cultures, or minimal M9 medium can be used to restrict growth for labeling purposes (3,4). Figure 3 shows the results of autoinduction in TB medium. While TB supports autoinduction of KRX, there was no significant difference in expression levels at different glucose and rhamnose concentrations, and protein expression rates reached a plateau at 16–24 hours.

Renilla luciferase expression was also not differentially regulated under the conditions tested (data not shown). Minimal M9 medium, which allows easy manipulation of the carbon energy source, is amenable to autoinduction using both early and late protocols (data not shown). Because autoinduction is regulated by sugars, the type of medium used will affect timing and optimal glucose and rhamnose conditions; therefore, optimization is recommended. Table 1 shows the energy sources in each of the media tested (5) and summarizes our results.

AUTOINDUCTION OF CRBPII

Based on glucose and rhamnose titration experiments using firefly and Renilla luciferases, we standardized early and late autoinduction methods in LB medium:

Early autoinduction: Dilute overnight cultures 1:100 in LB medium + 0.05% glucose + 0.1% rhamnose. Harvest cells after ~8 hours.

Table 1. Medium Composition Affects KRX Autoinduction Experiments.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Energy Source</th>
<th>Autoinduction Methods Supported</th>
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<tr>
<td>M9 minimal medium</td>
<td>20% carbon source (e.g., glucose)</td>
<td>Early and late autoinduction (data not shown)</td>
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<tr>
<td>Luria-Bertani (LB) medium</td>
<td>yeast extract 0.5% (w/v)</td>
<td>Early and late autoinduction (Figures 2 and 4)</td>
</tr>
<tr>
<td>Terrific Broth (TB)</td>
<td>yeast extract 2.4% (w/v)</td>
<td>No distinct early versus late autoinduction (Figure 3)</td>
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Figure 2. Autoinduction of firefly and Renilla luciferase expression by glucose and rhamnose titration in KRX. KRX transfected with pFN6A-luc (Panel A, firefly luciferase) and KRX transfected with pFN6A-hRluc (Panel B, Renilla luciferase) were grown overnight in LB medium. Overnight culture was diluted 1:100 in 3 ml of TB induction media (with supplements as indicated in Figure 2, Panel C) and incubated at 25 °C with shaking (time of dilution = 0 hours). Firefly luciferase activity was measured as described in Figure 2.

Figure 3. Titration of glucose and rhamnose did not affect firefly luciferase expression in Terrific Broth. KRX transfected with pFN6A-luc were grown overnight in LB medium. Overnight culture was diluted 1:100 in 3 ml of TB induction media (with supplements as indicated in Figure 2, Panel C) and incubated at 25 °C with shaking (time of dilution = 0 hours). Firefly luciferase activity was measured as described in Figure 2.

Adding glucose to KRX cultures supplies cells with a rich, consumable energy source that supports growth but delays induction by rhamnose. By titrating glucose in the induction medium, the time of protein induction can be regulated.
Because autoinduction is regulated by sugars, the medium used will affect timing and optimal glucose and rhamnose conditions; therefore, optimization is recommended.

Late autoinduction: Dilute overnight cultures 1:100 in LB medium + 0.15% glucose + 0.1% rhamnose. Harvest cells after ~16–24 hours.

We used both methods to express an additional protein, human cellular retinol binding protein II (CRBPII), in the KRX strain. Figure 4, Panel A, clearly shows induction of CRBPII by 8 hours in early autoinduction medium. Figure 4, Panel B, shows the effect of late autoinduction medium on CRBPII expression, as significant protein levels are not seen until 24 hours.

CONCLUSIONS

Autoinduction of protein expression is made possible by tight regulation of recombinant protein expression in KRX cells. The procedure is simple and only requires addition of glucose and rhamnose to typical E. coli growth media. Adding glucose to cultures supplies cells with a rich, consumable energy source that supports growth but delays induction by rhamnose. By titrating the amount of glucose in the induction medium, the time of protein induction can be regulated: low concentration for early induction, high concentration for late induction. Optimization may be required for other proteins and culture volumes, particularly with media other than those used here. These autoinduction protocols eliminate the need to monitor culture density prior to induction; overnight cultures can be diluted directly in the autoinduction medium and harvested 8–24 hours later. These protocols also highlight the tight control of protein expression in the KRX strain. This is advantageous when optimizing expression of toxic or difficult-to-express proteins.

REFERENCES


ORDERING INFORMATION

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<th>Product</th>
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<td>50 g</td>
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