TKB1 Competent Cells

Instruction Manual

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Revision D.0

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TKB1 Competent Cells

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MATERIALS PROVIDED

Materials provided[a]	Quantity
TKB1 competent cells (red-orange tubes)[b]	5 × 200-µl aliquots
pUC18 control plasmid DNA (0.1 ng/µl in TE buffer[c])	10 µl
1.42 M β-mercaptoethanol	25 µl

[a] Provides enough reagents for 10 reactions.

[b] Transformation efficiency is $>5 \times 10^5$ cfu/µg of pUC18 DNA. We guarantee the transformation efficiency for these competent cells when used according to the specifications outlined in this instruction manual.

[c] See *Preparation of Media and Reagents*.

STORAGE CONDITIONS

Store cells immediately at –80°C

Do not place the cells in liquid nitrogen

ADDITIONAL MATERIALS REQUIRED

14-ml BD Falcon® polypropylene round-bottom tubes (BD Biosciences Catalog #352059)

LB agar plates[§] with 12.5 µg/ml of tetracycline and selecting antibiotic for plasmid encoding the target protein gene

Antibiotics

- Ampicillin stock (50 mg/ml in H_2O)
- Tetracycline stock [12.5 mg/ml in 50% (v/v) ethanol]

Indoleacrylic acid [2.5 mg/ml in 95% (v/v) ethanol]

TK induction medium[§]

LB broth[§] for target gene expression

IPTG (isopropyl-β-D-thio-galactopyranoside) inducer for target gene expression (stock is 100 mM in H_2O) (Catalog #300127).

[§] See *Preparation of Media and Reagents.*

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INTRODUCTION

The TKB1 competent cells are a tyrosine kinase (TK) derivative of the BL21(DE3) strain [*E. coli* B F^- *dcm ompT hsdS*(r_B^- m_B^-) *gal* λ(DE3)].[1–3] The BL21(DE3) TK strain, abbreviated TKB1, carries the gene for T7 RNA polymerase in the chromosome under the control of the *lacUV5* promoter. Induction of the polymerase with isopropyl-β-D-thio-galactopyranoside (IPTG) allows controlled protein expression of genes placed downstream of the T7 RNA polymerase binding site. The TKB1 strain harbors a plasmid-encoded, inducible tyrosine kinase gene (pTK). The TKB1 strain can be transformed with a plasmid (ColE1 origin) containing a DNA sequence encoding a phosphorylation target domain or protein. If the target protein is cloned as a fusion to an affinity tag, purification of large amounts of phosphorylated protein is relatively easy. The phosphorylated protein isolated from the TK strain can be used to screen expression libraries and to affinity purify or blot proteins that interact with the tyrosine-phosphorylated target.

HOST STRAIN AND GENOTYPE

For the *Escherichia coli* strain, the genes listed in the table below signify that the bacterium carries a mutant allele.

Host strain	References	Genotype
TKB1 strain[a]	2, 3	*E. coli* B F^- *dcm ompT hsdS*(r_B^- m_B^-) *gal* λ(DE3) [pTK Tet^r]

[a] This strain, a derivative of *E. coli* B, is a general protein expression strain that lacks both the *lon* protease and the *ompT* protease, which can degrade proteins during purification (see Reference 4).

TRANSFORMATION GUIDELINES

Important *Please read the following guidelines before proceeding with the* Transformation Protocol.

It is important to store the competent cells at –80°C to prevent a loss of efficiency. For best results, please follow the directions outlined in the following sections.

Storage Conditions

Competent cells are very sensitive to even small variations in temperature and must be stored at the bottom of a –80°C freezer. Transferring tubes from one freezer to another may result in a loss of efficiency. Competent cells should be placed at –80°C directly from the dry ice shipping container. Cells stored in this manner should retain their guaranteed efficiency for 6 months.

Aliquoting Cells

When aliquoting, keep the competent cells on ice at all times. It is essential that the 14-ml polypropylene tubes are placed on ice before the cells are thawed and that the cells are aliquoted directly into the prechilled tubes. It is also important to use at least 100 µl of competent cells/transformation. Using a smaller volume will result in lower efficiencies.

Use of BD Falcon® Polypropylene Tubes

It is important that 14-ml BD Falcon® polypropylene round-bottom tubes (BD Biosciences Catalog #352059) are used for the transformation protocol, since other tubes may be degraded by the β-mercaptoethanol used in step 3 of the *Transformation Protocol*. In addition, the incubation period during the heat-pulse step is critical and has been optimized for the thickness and shape of the 14-ml BD Falcon polypropylene round-bottom tubes.

Use of β-Mercaptoethanol

β-Mercaptoethanol has been shown to increase transformation efficiency two- to threefold. This kit includes prediluted β-mercaptoethanol, which is ready to use. Using the β-mercaptoethanol within 3 months is recommended. Use 1.7 µl of the β-mercaptoethanol provided or a fresh 1:10 dilution (stock solution 14.2 M)/100 µl of cells.

Quantity of DNA Added

Add 1–5 µl of DNA for a final concentration of 10–100 ng of DNA in the transformation.

Length of the Heat Pulse

There is a defined "window" of highest efficiency resulting from the heat pulse in step 8 of the *Transformation Protocol*. Optimal efficiencies are observed when cells are heat-pulsed for 45–50 seconds. Heat-pulsing for at least 45 seconds is recommended to allow for slight variations in the length of incubation. Efficiencies decrease sharply when incubating for <45 seconds or for >60 seconds.

TRANSFORMATION PROTOCOL

1. Thaw the TKB1 competent cells on ice.

2. Gently mix the competent cells by hand. Aliquot 100 µl of the competent cells into a prechilled 14-ml BD Falcon polypropylene round-bottom tube.

3. Add 1.7 µl of β-mercaptoethanol provided with this kit or a fresh 1:10 dilution (stock 14.2 M) of β-mercaptoethanol [diluted in distilled water (dH_2O)] to the 100 µl of competent cells, giving a final concentration of 25 mM.

4. Swirl the contents of the tube gently. Incubate the polypropylene tube on ice for 10 minutes, swirling the tube gently every 2 minutes.

5. Add 10–100 ng (1–5 µl) of DNA to the polypropylene tube and swirl gently. As an optional transformation efficiency control, add 1 µl of the pUC18 control plasmid to another 100-µl aliquot of the competent cells and swirl gently.

6. Incubate the two polypropylene tubes on ice for 30 minutes.

7. Preheat SOC medium[§] in a 42°C water bath for use in step 10.

8. Heat-pulse the tubes in a 42°C water bath for 45 seconds. The duration of the heat pulse is critical for obtaining the highest efficiencies.

9. Incubate the tubes on ice for 2 minutes.

10. Add 0.9 ml of 42°C SOC medium and incubate the tubes at 37°C for 1 hour with shaking at 225–250 rpm.

11. When plating the transformed cells, use a sterile spreader to plate ≤200 µl of the transformation mixture on the appropriate antibiotic agar plates.[§,‖] For the control transformations, use LB–ampicillin–tetracycline agar plates.[§]

    Note *The cells may be concentrated by centrifuging at 200 × g for 3–5 minutes at 4°C if desired. Resuspend the pellet in 200 µl of SOC medium and plate immediately.*

    Plate 200 µl of the cells transformed with the control pUC18 plasmid.

    Proceed to the *Induction Protocol*.

[§] See *Preparation of Media and Reagents*.

[‖] When spreading bacteria onto the plate, tilt and tap the spreader to remove the last drop of cells. For the competent cells, if plating <100 µl of the transformation mixture, plate into a 200-µl pool of SOC medium. If plating ≥100 µl, the cells can be spread directly onto the plates.

Expected Results

Bacterial strain	Amount plated	Expected colony number (with the control)	Efficiency (cfu/µg of pUC18 DNA)
TKB1 strain	200 µl	>100	$>5 \times 10^5$

INDUCTION PROTOCOL

Introduction

A two-step protocol has been shown to effectively produce large amounts of phosphorylated fusion protein. Expression of the kinase **target** gene is induced, and the target protein is allowed to accumulate before expression of the kinase gene is induced. Expression of the kinase target gene is induced by addition of IPTG to growing cultures. We recommend a final concentration of 0.4 mM IPTG for constructions carrying the "plain" T7 promoter, or of 1 mM IPTG for vectors having the T7 *lac* promoter.

1. Select a single colony from a freshly streaked plate and inoculate LB broth containing the appropriate antibiotics [tetracycline (12.5 µg/ml) + target plasmid selection antibiotic]. For good aeration, add medium up to only 20% of the total flask volume.

   The BL21(DE3) strain and its TK derivative, TKB1, grow rather slowly. The following steps illustrate a convenient method to generate the seed culture:

   a. Inoculate a single colony into 2 ml of LB broth supplemented with antibiotics and incubate with shaking at 37°C until the OD_{600} reaches 0.6–1.0. This step may take 6–8 hours.

   b. Chill this culture on ice for 10 minutes and store overnight at 4°C.

2. Collect the cells the following morning by centrifugation and inoculate 50 ml of LB broth.

3. Grow the culture at 37°C to an OD_{600} of 0.4–1.0 (We recommend an OD_{600} of 0.6). Remove a sample for the uninduced control and add IPTG to 0.4 mM (for T7 promoter constructs) or 1.0 mM (for T7 *lac* promoter constructs) to the induced culture. Continue to incubate both cultures for 2–3 hours.

   Note *Optimal induction conditions should be determined for your target gene. Production of the target protein can be monitored over time after induction by SDS–PAGE analysis of extracts prepared from cultures harvested after using different induction parameters.*

4. Measure the optical density of the culture and collect the cells by centrifugation at 2000 × *g*. Resuspend the cells in the TK induction medium to an OD_{600} of 0.5.

5. Incubate the TK-induced cultures for 2 hours at 37°C with shaking.

6. Harvest the cells by centrifugation at 2000 × *g* for 10 minutes.

Analysis of Extracts

Phosphorylation of the target gene can be evaluated by immunoblotting using an antiphosphotyrosine antibody. After resolution of *E. coli* extract proteins or purified target proteins by SDS–PAGE, proteins can be electrotransferred to a membrane and blotted with any one of several commercially available antiphosphotyrosine antibodies.

PREPARATION OF MEDIA AND REAGENTS

LB Agar (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Adjust pH to 7.0 with 5 N NaOH
Add deionized H_2O to a final volume of 1 liter
Autoclave
Pour into petri dishes (~25 ml/100-mm plate)

LB–Tetracycline Agar (per Liter)

1 liter of LB agar
Autoclave
Cool to 55°C
Add 12.5 mg of filter-sterilized tetracycline
Pour into petri dishes (~25 ml/100-mm plate)
Store plates in a dark, cool place or cover plates with foil if left out at room temperature for extended time periods as tetracycline is light-sensitive

LB–Ampicillin–Tetracycline Agar (per Liter)

(Use for reduced satellite colony formation)
1 liter of LB agar
Autoclave
Cool to 55°C
Add 100 mg of filter-sterilized ampicillin
Add 12.5 mg of filter-sterilized tetracycline
Pour into petri dishes (~25 ml/100-mm plate)

LB–Tetracycline Broth (per Liter)

1 liter of LB broth
Autoclave
Cool to 55°C
Add 12.5 mg of filter-sterilized tetracycline
Store broth in a dark, cool place as tetracycline is light-sensitive

LB Broth (per Liter)

5 g of NaCl
10 g of tryptone
5 g of yeast extract
Adjust to pH 7.0 with 5 N NaOH
Add deionized H_2O to a final volume of 1 liter
Autoclave

TE Buffer

10 mM Tris-HCl (pH 7.5)
1 mM EDTA

(Table continues on the next page)

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TK Induction Medium

Modified 5× M9 Medium (per Liter)

30 g of dibasic sodium phosphate (Na_2HPO_4)
15 g of monobasic potassium phosphate (KH_2PO_4)
5 g of ammonium chloride (NH_4Cl)
2.5 g of NaCl
15 mg $CaCl_2$
Autoclave

SOB Medium (per Liter)

20.0 g of tryptone
5.0 g of yeast extract
0.5 g of NaCl
Add deionized H_2O to a final volume of 1 liter
Autoclave
Add 10 ml of filter-sterilized 1 M $MgCl_2$ and 10 ml of filter-sterilized 1 M $MgSO_4$ prior to use

SOC Medium (per 100 ml)

Note *This medium should be prepared immediately before use.*

2 ml of filter-sterilized 20% (w/v) glucose or 1 ml of filter-sterilized 2 M glucose
SOB medium (autoclaved) to a final volume of 100 ml

1× TK Induction Medium (per Liter)

200 ml of modified 5× M9 medium
1 ml of 1 M $MgSO_4 \cdot 7H_2O$ (autoclave)
10 ml of 20% (w/v) glucose (filter sterilize)
5 ml of 20% casamino acids (filter sterilize)
0.1 ml of 0.5% thiamine-HCl (filter sterilize)
4 ml of 2.5 mg/ml indoleacrylic acid stock
1 ml of 50 mg/ml ampicillin [antibiotic selection for **target plasmid** (filter sterilize the stock solution and store at −20°C)
1 ml of 12.5 mg/ml tetracycline [mix tetracycline in 50% (v/v) ethanol and store at −20°C]
Dilute to 1 liter with sterile water

REFERENCES

1. Studier, F. W. and Moffatt, B. A. (1986) *J Mol Biol* 189(1):113-30.
2. Weiner, M. P., Anderson, C., Jerpseth, B., Wells, S., Johnson-Browne, B. *et al.* (1994) *Strategies* 7(2):41–43.
3. Simcox, M. E., Huvar, A., Simcox, T. and Vega, Q. (1994) *Strategies* 7(3):68–69.
4. Grodberg, J. and Dunn, J. J. (1988) *J Bacteriol* 170(3):1245-53.

ENDNOTES

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.