TurboCells® BL21

Chemically Competent *E. coli*

Genlantis
A division of Gene Therapy Systems, Inc.

Cat. #	Contents	Quantity
C302020	TurboCells® BL21(DE3) Chemically Competent *E. coli*	20 x 50 µl
	TurboCells® Transformation Buffer	5 ml
	pUC19 Positive Control Plasmid	20 µl (10pg/µl)
C303020	TurboCells® BL21(DE3)pLysS Chemically Competent *E. coli*	20 x 50 µl
	TurboCells® Transformation Buffer	5 ml
	pUC19 Positive Control Plasmid	20 µl (10pg/µl)

Shipping	Shipped on Dry Ice.
Storage	Store cells at -70°C; store TurboCells Transformation Buffer at room temperature. Stable for 6 months.

Related Products	Catalog #
EZ-Spread ™ Beads, Single-Use Tubes, 50 tubes	C400050
EZ-Spread ™ Beads, Dispenser Bottle	C400100
TurboCells® Chemically Competent *E. coli*	C300020
TurboCells® F′ Chemically Competent *E. coli*	C301020

Introduction: TurboCells® BL21(DE3) and TurboCells® BL21(DE3)pLysS Chemically Competent *E. coli* incorporate a unique 3-minute transformation protocol with efficient transgene expression from T7 promoter-based vectors. Both strains contain the λDE3 lysogen, which expresses T7 RNA polymerase under the control of a *lac*UV5 promoter. Upon addition of IPTG, the expression of the T7 RNA polymerase is induced, which allows for high-level protein expression from T7 promoter-based expression vectors (*e.g.*, pET). Additionally, BL21(DE3)pLysS contains the pLysS plasmid which produces T7 lysozyme, an inhibitor of T7 RNA polymerase, to reduce basal level expression of proteins that are toxic in *E. coli*.

Unlike other commercially available BL21 strains, the TurboCells BL21(DE3) and TurboCells BL21(DE3)pLysS allow efficient transformation without the need for a lengthy transformation reaction or hour-long recovery after heat shock. In addition, with TurboCells, you can perform the heat shock step at 37ºC, eliminating the inconvenient 42ºC water bath. TurboCells Competent *E. coli* are great for routine protein expression experiments. The fast and easy protocol also makes them ideal for high-throughput applications.

When using the 3-minute transformation protocol, you can achieve transformation efficiencies of up to 5 x 10^5 cfu/µg of supercoiled DNA, which is more than sufficient for most protein expression experiments. When using the traditional 1.5 – 2 hour transformation protocol with the TurboCells BL21(DE3) and TurboCells BL21(DE3)pLysS Competent *E. coli*, greater transformation efficiencies can be achieved.

For optimal transformation results and your convenience, TurboCells BL21(DE3) and TurboCells BL21(DE3)pLysS Chemically Competent *E. coli* are supplied in single-use 50 µl aliquots. This format avoids efficiency-robbing freeze-thaw cycles and wasted cells.

TurboCells® BL21(DE3): F- *ompT hsd*S_B (r_{B^-} m_{B^-}) *gal dcm* (DE3)	
TurboCells® BL21(DE3)pLysS: F- *ompT hsd*S_B (r_{B^-} m_{B^-}) *gal dcm* (DE3) pLysS (Cam^R)	
DE3	Encodes T7 lysogen for T7 RNA polymerase for high-level transcription
pLysS	Reduced basal expression of the T7 RNA polymerase. This reduces basal expression of toxic transgenes
CamR	Chloramphenical resistance; enables maintenance of the pLysS plasmid
ompT	Deficient in the OmpT protease, resulting in a higher yield of intact recombinant proteins
hsd SB (rB- mB-)	Improved cloning efficiencies and representations of methylated DNA

METHODS AND PROCEDURES

A. Transformation Protocol

1. Thaw one tube of the TurboCells competent cells on ice.
2. Transfer to pre-chilled Falcon 2059 tube.
3. Add 5 to 50 ng of DNA, in a volume of 1 to10 µl (or add 1 µl of 500 pg/µl of pUC19 control plasmid) to the cells; mix by tapping gently and incubate on ice for 3 to 5 minutes.
4. Heat shock the mix at 37ºC for 60 seconds.
5. Dilute the transformation reaction with 200 µl TurboCells Transformation Buffer and spread 25–100 µl of transformed cells on 37°C pre-warmed LB/Agar plates containing the appropriate antibiotic selection (e.g. 50 µg/ml ampicillin). We recommend using EZ-Spread™ Plating Beads (Cat. No. C400050 & C400100) to obtain optimal plating results.
6. If using <100 µl of transformed cells, add enough TurboCells, and enough TurboCells Transformation Buffer to make up 100 µl for ease of spreading.

   IMPORTANT: If kanamycin is used for antibiotic selection, add 0.25 ml room temperature SOC medium and incubate at 37°C with horizontal shaking at 225 rpm for 1 hour in an air incubator, prior to adding 600 µl TurboCells Transformation Buffer.
7. Incubate overnight at 37ºC.

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Transformation Notes

a. If maximum numbers of colonies are desired, replace Step 4 with the following: collect cells by spinning in a microfuge for 10 seconds. Resuspend cell pellet in 50 µl TurboCells Transformation Buffer and spread on an agar plate containing antibiotic.

b. If satellite colonies appear on the plates after overnight incubation, we recommend to: 1) reduce the volume of transformation reaction used for plating, or 2) if ampicillin is used, replace it with a more stable homologue such as carbenicillin (disodium salt), or 3) increase the antibiotic concentration in the plate.

c. It is not necessary to dilute your ligation mix with TE. In our tests, up to 10 µl of undiluted ligation mix could be used with the TurboCells® BL21(DE3) or TurboCells® BL21(DE3)pLysS without significantly compromising the transformation efficiency.

d. To increase transformation efficiencies and obtain higher number of colonies, we recommend the following traditional protocol:
   1. Thaw one tube of the TurboCells competent cells on ice (10-15 minutes).
   2. Add 1-10 µl of ligation mix to the cells; mix gently and incubate on ice for 15 to 30 minutes.
   3. Heat the mix at 42ºC for 45 seconds.
   4. Add 0.25 ml room temperature SOC medium and incubate at 37ºC for 1 hour in an air incubator. Shaking tubes horizontally at 225 rpm is recommended for the best efficiency.
   5. Dilute the transformation reaction if necessary and spread 100 µl of transformed cells on LB/Agar plates containing the appropriate antibiotic selection (e.g. ampicillin or kanamycin).
   6. Incubate overnight at 37ºC.

e. Transformation efficiencies for ligation of inserts to vectors will be between 5-10 fold lower than supercoiled plasmids.

f. To test the efficiency of competent cells using the provided supercoiled pUC19 plasmid DNA, please use the following protocol:
   1. Transform 1 µl (500 pg) pUC19 into 50 µl of competent cells. Incubate on ice for 3 minutes.
   2. Heat shock the mix at 37ºC for 60 seconds. Place on ice immediately and incubate for 2 minutes.
   3. Dilute the transformation reaction 10 fold with TurboCells™ Transformation Buffer and plate 50 µl on a LB agar plate containing 50 µg/ml ampicillin.
   4. Incubate overnight at 37ºC and count colonies. Calculate transformation efficiency as follows:

$$\frac{\text{Number of Colonies}}{500 \text{ pg pUC19}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{50 \text{ µl}}{50 \text{ µl plated}} \times 10^{\dagger} = \text{CFU/µg}$$

† 10 is the dilution factor

B. Example Protocol for Protein Expression

1. Following transformation, pick 3 or 4 individual colonies, and with each one, inoculate 1 - 50 ml aliquots of LB medium containing the appropriate antibiotic to select for the expression vector.
   IMPORTANT: If using TurboCells™ BL21(DE3)pLysS, include 40 µg/ml chloramphenicol in the LB medium to maintain the pLysS plasmid.
   TIP: Perform a fresh transformation of BL21(DE3)pLysS cells before each induction experiment.
2. Incubate each aliquot overnight with shaking at 37°C.
3. The following day, transfer a 50 µl aliquot of each culture to 1 ml of fresh LB medium without antibiotics, and incubate for approximately 2 hours at 37ºC until OD600 reaches 0.4–0.9.
4. Remove a 100 µl aliquot of each culture and place on ice as a non-induced negative control.
5. With the remaining 950 µl of each culture, induce by adding IPTG to a final concentration of 1.0 mM, and culture for 2-3 hours. A range of IPTG concentrations (0.4 mM – 1.0 mM) and time points are recommended to determine optimal conditions for expression of your protein.
6. After the end of the induction period, protein expression may be analyzed by Western blot, SDS-PAGE, or enzyme assay.
7. It is possible to save the transformed TurboCells BL21(DE3) cells if desired. Simply isolate the cells by centrifugation at 5000 x g for 5 minutes. Remove the supernatant and store the cells as a frozen pellet at -70ºC.

Protein Expression Notes

a) The above section provides general guidelines for performing protein expression in TurboCells™ BL21(DE3) and TurboCells™ BL21(DE3)pLysS Chemically Competent E. coli using T7 RNA polymerase-based expression plasmids. These guidelines are optional, and they may be modified or discarded as required by your particular application.

b) The above section provides general guidelines for performing protein expression in TurboCells™ BL21(DE3) and TurboCells™ BL21(DE3)pLysS Chemically Competent E. coli using T7 RNA polymerase-based expression plasmids. These guidelines are optional, and they may be modified or discarded as required by your particular application.

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c) For expression of proteins known to be non-toxic in E. coli, we recommend using the TurboCells® BL21(DE3), as this strain provides the highest levels of protein expression in most cases.

d) For expression of proteins known to be toxic in E. coli, we recommend using the TurboCells BL21(DE3)pLysS strain. This strain contains the pLysS plasmid, which encodes T7 lysozyme. T7 RNA polymerase is significantly inhibited by T7 lysozyme, thus "leakage" (i.e., basal level expression) of toxic recombinant proteins in uninduced cells is significantly reduced.

e) We recommend that TurboCells BL21(DE3) and TurboCells BL21(DE3)pLysS be used **for expression only.** To maintain and propagate your T7 expression vector, use an E. coli strain that does not contain the T7 RNA polymerase, e.g. TurboCells Chemically Competent E. coli, such as:

Product Name	Cat. No.
TurboCells® Chemically Competent *E. coli*	C300020
TurboCells® F′ Chemically Competent *E. coli*	C301020

Troubleshooting Guide

Problem	Possible Causes	Recommended Solutions
No or very few colonies on agar plate after transformation	Gene of interest is toxic in *E. coli*	Do Use TurboCells™ BL21(DE3)pLysS Competent *E. coli* to reduce basal level protein expression.
	Competent cells are dead due to improper storage or shipping conditions	The efficiency of competent cells can be verified using a plasmid such as pUC19. Make sure you store the TurboCells™ Competent *E. coli* at –70°C.
	Wrong antibiotic selection.	Double-check that correct antibiotic selection was used.
No or very little protein expression detected after induction.	Poor vector design	Check if expression cassette is in-frame with promoter.
	Induction time and IPTG concentration not optimized.	Optimize IPTG to a final concentration of 0.4 – 1.0 mM, and culture time for 2-3 hours. Also, temperature may be optimized between 25-37ºC.

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