Intact Genomics®

# BL21

# Chemically Competent Cells

| Catalog # | Package Size |
|---|---|
| 1041-06 | 6x50 µl |
| 1041-12 | 12x50 µl |
| 1042-12 | 6x100 µl |
| 1042-24 | 12x100 µl |

## Description

Intact Genomics BL21 chemically competent cells are suitable for transformation and routine protein expression from non-T7 vectors.

## Specifications

| | |
|---|---|
| Competent cell type: | Chemically competent |
| Derivative of: | BL21 |
| Species: | *E. coli* |
| Format: | Tubes |
| Transformation efficiency: | ≥1 x $10^8$ cfu/µg pUC19 DNA |
| Blue/white screening: | Yes |
| Shipping condition: | Dry ice |

## Reagents Needed for One Reaction

| | |
|---|---|
| BL21 chemically competent cells: | 50 µl |
| DNA (or pUC19 Control, 10 pg/µl): | 1 µl |
| Recovery medium: | 1 ml |

## Storage

| | |
|---|---|
| BL21 competent cells: | -80 ºC |
| pUC19 control DNA: | -20 ºC |
| Recovery medium: | 4 ºC |

## Genomic Features

BL21 chemically competent cells have the following features:

- Widely used host background.
- Routine non-T7 vector expression.
- Deficient in both lon (1) and ompT proteases.
- Resistant to phage T1 (fhuA2).

## Genotype

*F- dcm ompT hsdS(rB- mB-) gal [malB+]$_{K-12}$(λS)*

## Quality Control

Transformation efficiency is tested by using the pUC19 control DNA supplied with the kit and using the high efficiency transformation protocol listed below. Transformation efficiency should be ≥1x$10^8$ CFU/µg pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

## General Guidelines

Follow these guidelines when using BL21 chemically competent *cells*.

- Handle competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting.
- Thaw competent cells on ice, and transform cells immediately following thawing. After adding DNA, mix by tapping the tube gently. Do not mix cells by pipetting or vortexing.

## Calculation of Transformation Efficiency

Transformation Efficiency (TE) is defined as the number of colony forming units (cfu) produced by transforming 1µg of plasmid into a given volume of competent cells.

TE = Colonies/µg/Dilution

Transform 1 µl of (10 pg/µl) pUC19 control plasmid into 50 µl of cells, add 950 µl of Recovery Medium. Dilute 10 µl of this in 990 µl of Recovery Medium and plate 50 µl. Count the colonies on the plate the next day. If you count 100 colonies, the TE is calculated as follows:

Colonies = 100
µg of DNA = 0.00001
Dilution = 50/1000 x 10/1000 = 0.0005
TE = 100/.00001/.0005 = 2.0x$10^{10}$

## High Efficiency Transformation Protocol

Use this procedure to transform BL21 chemically competent cells. We recommend verifying the transformation efficiency of the cells using the pUC19 control DNA supplied with the kit. Do not use these cells for electroporation.

1) Remove competent cells from the -80 °C freezer and thaw completely on wet ice (10-15 minutes).
2) Aliquot 1-5 µl (1 pg-100 ng) of DNA to the chilled microcentrifuge tubes on ice.
3) When the cells are thawed, add 50 µl of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pUC19 control, add 1 µl of (10 pg/µl)
   DNA to a chilled microcentrifuge tube, prior to adding 50 µl of cells. Mix well by tapping. Do not pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.
4) Incubate the cells with DNA on ice for 30 minutes.
5) After 30 minute ice incubation, heat shock the cells at 42 °C for 45 seconds.
6) Transfer the tubes to ice for 2 minutes.
7) Add 950 µl of Recovery Medium or any other medium of choice to each tube.
8) Incubate tubes at 37 °C for 1 hour at 210 rpm.
9) Spread 50 µl to 200 µl from each transformation on Pre-warmed selection plates. We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, plate 50 µl on an LB plate containing 100 µg/ml ampicillin. Use sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
10) Incubate the plates overnight at 37 °C.

## 5 Minute Transformation Protocol

The following procedure results in only ~10% of the transformation efficiency as the protocol listed above.

1) Remove competent cells from the -80 °C freezer and thaw in your hand.
2) Aliquot 1-5 µl (1 pg-100 ng) of DNA to the microcentrifuge tubes. ***Do not*** pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.
3) Incubate the cells with DNA on ice for 2 minutes.
4) After 2 minute ice incubation, heat shock the cells at 42 °C for 45 seconds.

5) Transfer the tubes to ice for 2 minutes.
6) Add 950 µl of Recovery Medium at room temperature or any other medium of choice to each tube. Immediately spread 50 µl to 200 µl from each transformation on pre-warmed selection plates. We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, plate 50 µl on an LB plate containing 100 µg/ml ampicillin. Use sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
7) Incubate the plates overnight at 37 °C.