



## Eco™ Competent E Coli Cell User Manual

<b>Chemical Competent cells</b>	<b>Catalog Number</b>	<b>Contents</b>	<b>Note</b>
Eco™_DH5a_Cloning cells	CC01	20 x 50ul	
Eco™_DH5a_Lenti cells	CC02	20 x 50ul	
Eco™_BL21(DE3)_ cells	CC03	20 x 50ul	
Eco™_BL21(pLys)_ cells	CC03p	20 x 50ul	
Eco™_BL21_Toxic_ cells	CC04	20 x 50ul	
96-well plate competent cells	CC-plate	50ul/well	Pick cell type
<b>Electro Competent cells</b>	<b>Catalog Number</b>	<b>Contents</b>	
Eco™_DH10_Electro cells	EC01	20 x 50ul	

### Storage:

Cells were shipped on dry ice. Upon received, they should be stored at -80°C, and good for 6 month when store appropriately. Do not thaw until use.

### Product Description:

GenTarget Inc's Eco™ Competent Cells were pre-made for chemical transformation or electroporation. Chemical Transformation efficiencies typically range from  $10^8$ – $10^9$  transformants/μg of pUC19 DNA. Electroporation efficiency is  $10^{10}$  transformants / per μg of pUC19 DNA. For cDNA library and ligation transformation, the efficiencies may be lower by 10 ~100 folds, which is normal.

Competent cells are supplied as a pack of 20 x 50 μl/tube aliquots, or in a 96-well format. All competent cells were tested on a lot-to-lot basis to ensure the product performance and quality.

### To select competent types:

<b>Application</b>	<b>Competent cells to use</b>	<b>Cat#</b>
General cloning	Eco™_DH5a_Cloning cells	<b>CC01</b>
Lentiviral or Retroviral cloning	Eco™_DH5a_Lenti cells	<b>CC02</b>
General protein expression	Eco™_BL21(DE3)_ cells	<b>CC03</b>
Toxic protein expression	Eco™_BL21(pLys)_ cells	<b>CC03p</b>
Extreme toxic protein expression, or get protein expressed out of inclusion body	Eco™_BL21_Toxic_ cells	<b>CC04</b>
cDNA library construction	Eco™_DH10_Electro cells	<b>EC01</b>
High through-put cloning	96-well plate competent cells	<b>CC-plate</b>



## **Eco™\_DH5 $\alpha$ \_Cloning cells:**

Commonly used for high quality plasmid preparation in general cloning and cDNA library construction with resistant to bacteriophage infection. It is suitable for Blue/white screening of transformants on selective plates containing Xgal. **Genotypes:** endA1 hsdR17(rk-, mk+) recA1 phoA supE44 thi-1 gyrA relA1 tonA(mut) F- $\phi$ 80dlacZ $\Delta$ M15  $\Delta$ (lacZYA-argF)U169 deoR

## **Eco™\_DH5 $\alpha$ \_Lenti cells:**

Genetically modified strain. Used for transformation of unstable plasmid containing direct repeats. It is best suitable for Retroviral / Lentiviral vector propagation. **Genotypes:** endA1 hsdR17(rk-, mk+) recA1 phoA supE44 thi-1 gyrA relA1 tonA(mut) F- $\phi$ 80dlacZ $\Delta$ M15  $\Delta$ (lacZYA-argF)U169 deoR StrR

## **Eco™\_BL21(DE3)\_cells:**

For used in high-level gene expression and production of recombinant proteins in bacterial systems for any T7-based expression vector (for example, pET vectors). Strains carry a copy of the T7 RNA polymerase under control of the IPTG inducible lacUV5 promoter. **Genotypes:** F- ompT hsdSB (rB-mB-) gal dcm rne131 (DE3)

## **Eco™\_BL21(DE3)pLys\_cells:**

For used in high-level gene expression and production of recombinant proteins in bacterial systems for any T7-based expression vector (for example, pET vectors). Strains carry a copy of the T7 RNA polymerase under control of the IPTG inducible lacUV5 promoter. It also contains a plasmid expressing T7 lysozyme which inhibits T7 RNA polymerase for reducing the basal expression of target. **Genotypes:** F- ompT hsdSB (rB-mB-) gal dcm rne131 (DE3) pLys (CmR)

## **Eco™\_BL21\_Toxic:**

The strain carries the T7 RNA polymerase gene in its chromosome. The expression of T7 RNA polymerase is tightly regulated by L-arabinose so the basal expression is kept at minimal. **Genotypes:** F- araB::T7RNAP ompT hsdSB (rB-mB-) gal dcm

## **Eco™\_DH10\_Electro competent cells:**

Used for cloning of both prokaryotic and eukaryotic genomic DNA, is capable of Blue/White screening, and suitable for construction of



gene banks or for generation of cDNA libraries using electroporation. **Genotype:** F-  $\phi$ 80lacZ  $\Delta$ M15 galU galK  $\lambda$  - rpsL nupG tonA  $\Delta$ lacX74 recA1 endA1 araD139  $\Delta$ (ara, leu)7697 mcrA  $\Delta$ (mrr-hsdRMS-mcrBC)

## Chemical Transformation Protocol:

1. After competent cells completely thaw on ice, add 1-5  $\mu$ l plasmid DNA to a tube of thawed Competent cells, mix by gently tapping the tube. Avoid repeated pipetting;
2. Set the transformation mixture(s) in an ice bath (0°C) for 5-30 minutes.
3. Heat shock mixture(s) in 42°C water bath for 40 seconds;
4. After heat-shock, incubate the mixture(s) on ice for 1 min;
5. Add 250ul SOC medium, incubate at 37°C, shaking for 30min-60min;
6. Spread 10-150  $\mu$ l onto a **pre-warmed** culture plate;
7. Incubate the plate at 37°C overnight.

## Electroporation Protocol:

1. Precipitate DNA and resuspend in TE buffer to remove any salts in the samples. Set DNA on wet-ice.
2. Thaw Eco<sup>TM</sup>\_DH\_Lib Electro competent cells on wet ice. When cells are thawed, add 1  $\mu$ l of DNA to each cell tube (the DNA amount should not exceed 200 ng).
3. Pipette the cell/DNA mixture into a chilled 0.1 cm cuvette.
4. Electroporation using the following conditions: 2.0 kV, 200  $\Omega$ , 25  $\mu$ F.
5. Add 1 ml of S.O.C. medium to the cells in the cuvette and transfer it to a 15 ml culture tube. Shake at 225 rpm (37°C) for 1 hour.
6. Dilute cells with S.O.C. medium (make multiple dilution to get desired colony count on the plate). Spread 50  $\mu$ l of the dilution on pre-warmed LB plates containing appropriate antibiotic.
7. Incubate plates overnight at 37°C.

## Protein expression Protocol:

### For expression in Eco\_BL21\_Cells (Cat#: **CC03**):

- Pick 3 or 4 transformants and culture them in 5 ml LB medium containing the appropriate antibiotic to select for your expression plasmid. Grow at 37°C with shaking until the OD600 reaches 0.6 to 1.0.
- Inoculate LB medium containing the appropriate antibiotic to an OD600 of 0.05-0.1 (~1:20 dilution of the initial culture). Save glycerol stock of the clones using the initial culture. Once you have identified the clone that best expresses your protein, you



can use the glycerol stock to perform additional expression experiments.

- Grow the cultures until they reach mid-log (OD600= $\sim$ 0.4, 2 to 3 hours).
- Induce the cultures by adding IPTG to a final concentration of 1 mM (for the T7 based IPTG inducible vector) and culture for an additional 2-3 hours. Alternatively, you can use GenTarget's auto-induction **RichMeidum (Cat# [RM1000](#))** without adding of IPTG.
- Test the expression levels to determine which clone best expresses your protein of interest.

**NOTE: Eco\_BL21(DE3) Cells** is used for maximum expression of recombinant protein so some basal level expression was observed. If the protein is toxic and the basal level expression has to be minimized, please use the **Eco\_BL21\_Toxic** cells which is best for expression of toxic protein.

#### For expression in Eco\_BL21\_Toxic cells (Cat#: [CC04](#)) :

- Pick several transformants and culture them in 5 ml LB medium containing the appropriate antibiotic. Grow at 37°C with shaking until the OD600 reaches 0.6 to 1.0.
- Inoculate LB medium containing the appropriate antibiotic to an OD600 of 0.05-0.1 (~1:20 dilution of the initial culture). Save glycerol stock of the initial cultures if need.
- Grow the cultures until they reach OD600 =  $\sim$ 0.4 (should take 2 to 3 hours). If the expressed protein is extremely toxic, add glucose to the final concentration of 0.1% into the medium for further repress the basal expression level.
- Induce the cultures by adding L-arabinose to a final concentration of 0.2%, and if the expression vector's T7 RNA polymerase is IPTG inducible, add IPTG to a final concentration of 1 mM, and culture for an additional 2-3 hours. Alternatively, you can use GenTarget's auto-induction **RichMeidum (Cat# [RM1000](#))** without adding of IPTG.
- Test the expression levels to determine which clone best expresses your protein of interest.