ClearColi BL21(DE3) Competent Cells

Cat: C3740
Size: 10×100μL
Storage: Store at -70°C to avoid repeated freezing and thawing.

Product Parameters:
English name: ClearColi BL21(DE3) Competent Cells

Genotype:
F-ompT hsdSB (rB-mB -) gal dcm lon λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5]) msbA148 ΔgutQ ΔkdsD ΔlpxL ΔlpxM ΔpagP ΔlpxP ΔeptA

Strain Resistance:
Sensitive to ampicillin, kanamycin, spectacular, chloramphenicol, streptomycin, and tetracycline.

Product Components:

Components	Size
ClearColi BL21(DE3) Competent Cells	10×100μL
pUC19(0.1ng/μL)	5μL

Introduction:

Lipopolysaccharide(LPS) is a component of the outer membrane of almost all Gram-negative bacteria. LPS is recognized by TLR4/MD-2 on mammalian immune cells, which activates the NF-kappB signaling pathway, leading to the occurrence of the organism's inflammatory response. Residual LPS in E. coli genetically engineered products can greatly affect downstream experiments. The outer membrane of ClearColi is the modified Lipid IVA. Compared with wild-type LPS, the lipid IVA lacks oligosaccharides and two secondary acyl chains. Lipid IVA does not induce the formation of activated hTLR4/MD-2 complex. It avoids endotoxin reaction in the LPS-TLR4/NF-kB signaling pathway. In addition, lipoid IVA, which does not contain oligosaccharides, is more easily removed from recombinant proteins.

ClearColi BL21(DE3) cells grew at about 50 percent the rate of normal BL21(DE3) cells. The transformed colony appears very small for the first 24 hours. It is suggested that the transformed plates should be cultured in a culture dish for 36-48 hours and then selected for the experiment. Before induction, longer growth time is needed to reach the desired cell density. pUC19 plasmid detected that the conversion efficiency of competent cells was greater than 10^5 cfu/μg.

Features:

Recombinant protein for expressing low endotoxin. This strain contains T7 RNA polymerase and E. coli RNA polymerase, which is suitable for the efficient expression of prokaryotic expression vectors containing T7 promoters(such as pET, etc.) and non-T7 promoter expression vectors(such as pGEX, pMal, pTrc, etc.) in the prokaryotic system.

Protocols:

1. Plasmid transformation steps

1) The competent cells are placed in an ice water bath to defreeze. After the cells are just defreeze, the target plasmid was added to the cells, dial the bottom of the tube with your finger, and mix gently;
2) Place in ice bath for 30min, do not shake;
3) Heat shock at 42℃ for 60s, do not shake;
4) Place in ice bath for 2min, do not shake;

5) Add 500μL sterile LB medium;
6) The culture was placed in a shaking table at 37°C, 150-200rpm for 60min.
7) Take 100-200μL bacterial solution and apply it on LB plate containing corresponding antibiotics. Put upside down in 37°C incubator for 36-48h.

2. Protein induction regimen

1) A single, well-grown colony was selected from the plate and inoculated into 10mL liquid LB medium containing appropriate antibiotics.
2) Oscillating culture overnight at 200-250rpm at 37°C.
3) Under sterile conditions, 8mL of overnight culture was inoculated into 200mL of LB culture containing appropriate antibiotics. Or determine the amount of inoculation by measuring OD600, and the final OD600 is about 0.1.
4) Oscillating culture at 200 to 250rpm at 37°C until OD600 reaches 0.6-0.8(about 4-5h).
5) IPTG was added until the final concentration was 0.4-1mM for induced expression of the protein. (2.38g IPTG was added to water by dissolving to prepare 1M IPTG solution, and the final volume was adjusted to 10mL. Sterilize by filtration prior to use). In order to determine the optimal concentration induced by IPTG to maximize the expression of the target protein, the concentration can be used with an IPTG gradient test.
6) Oscillatory induction culture at 37°C for 3-4h. In order to determine the optimal time for induction of the target protein, it is recommended to perform a time history experiment with induction time of 2-16h.
7) After induction, 5000×g centrifugation is performed for 10min to collect cells.
8) The total protein, supernatant and precipitated components of the lysate were analyzed by appropriate methods(such as Coomassie brilliant blue stain, Western-Blot or enzyme activity analysis), and the expression status of the product(soluble or insoluble expression) was clearly expressed.
9) According to the expression results and the characteristics of the target protein, a suitable protein purification program was developed.

Notes:

1. The ClearColi BL21(DE3) strain is slow growing and plate cultured at 37°C for between 36-48h.
2. The extracellular membrane of ClearColi BL21(DE3) was modified lipid IVA, which was suitable for growth in high salt medium, LB medium was commonly used, and no media such as SOB, 2×YT and SOC were used.
3. Competent cells should be defrozen in an ice bath and gently mixed after adding DNA. The volume of added DNA should be less than 1/10 of the cell volume.
4. If the biochemical reagents produced by our company are not specially marked, they are basically non-aseptic packaging. If used in cell experiments, please pre-treat them in advance.
5. Once it is prepared into a solution, please pack it separately and store it to avoid product failure caused by repeated freezing and thawing.
6. The product information is for reference only, if you have any questions, please call 400-968-6088 for consultation.
7. This product is for scientific use only. Do not use for medicine, clinical diagnosis or therapy, food or cosmetics. Do not store in ordinary residential areas.
8. For your safety and health, please wear a lab coat and wear disposable gloves and a mask.