ArcticExpress(DE3) Competent Cells

Cat: C3730
Size: 10×100μL/20×100μL
Storage: Store at -70°C to avoid repeated freezing and thawing.

Product Parameters:
English name: ArcticExpress(DE3) Competent cells

Genotype:
F $^-$ ompT hsdS(rB$^-$ mB $^-$) dcm$^+$ Tet^r gal λ(DE3) endA Hte (cpn10 cpn60 Gent^r)

Introduction:

ArcticExpress(DE3) cells are derived from the BL21-Gold strain, which belongs to the Lon and OmpT protease-deficient E. coli B strain. The gentamicin-resistant plasmid was able to stably express Cpn10 and Cpn60, two cryogenic molecular companions of the Marine bacterium Oleispira antarctica. These chaperones showed higher activity than Escherichia coli GroEL/GroES at lower temperatures (4-12 ° C), and could better promote the correct folding of recombinant proteins at low temperatures, potentially increasing the yield and activity of soluble recombinant proteins, and overcoming the misfolding and insolubility of recombinant proteins. This strain also has the λ phage DE3 gene region, which can express T7 RNA polymerase, which is suitable for the efficient expression of prokaryotic expression vectors(such as pET) containing T7 promoters. Non-t7 promoter expression vectors(such as pGEX, pMal, pTrc, etc.) can also be expressed in this strain. The receptor cells of ArcticExpress(DE3) were made by a special process, and the conversion efficiency of pUC19 plasmid was greater than 1×10^7 cfu/μg DNA.

Protocols:

1. Plasmid transformation steps

1) The competent cells are placed in an ice water bath to defreeze. After the cells are just defreeze, plasmid DNA or 5-10μL of the connection product was added to the cells, dial the bottom of the tube with your finger, and gently mix;
2) Place in the 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 SOC or LB medium;
6) The culture was placed in a shaking table at 37℃, 150-200rpm for 60min.
7) 50-100μl bacterial solution was taken and coated on LB plate containing 20μg/mL gentamicin and appropriate concentration of transformed plasmid resistant antibiotics. After the liquid was drained, the plate was turned upside down and cultured at 37℃ for 12-16h.

(**Plate scribing separation method:** After the recovery culture, centrifuge at 12000rpm for 30s, discard the supernatant, leave about 100μL of liquid, gently blow the bacteria with 200μL suction

head, take 10μL of suspended bacterial liquid into more drops on the plate, tilt the suction head, and use the side of the suction head to scribing the liquid dripping on the plate. This method can obtain more and larger monoclonal colonies.)

(**Rapid plasmid conversion step:** Shorten the time of step 2 to 5min, for ampicillin resistant plasmids, after step 4 is completed, it can be directly coated or striated on the ampicillin resistant LB plate. For other resistant plasmids, 60min of resuscitation was required.)

2. Protein expression Procedure

1) Single colonies were selected and inoculated into 5mL LB medium with antibiotics;
2) The bacteria were incubated at 37℃ and 200rpm to logarithmic growth stage(OD600=0.4-0.8).
3) IPTG was added until the final concentration was 0.4mM, and the bacteria were induced at 37℃ for 2-4h or at 16℃ overnight.

(**Low temperature induction method:** Shake the bacteria to about 1-2 OD at 37℃ at 200rpm, then cool the culture to 10-13℃, culture at low temperature for 15min to reach the equilibrium, add IPTG to the shaker, and the final concentration reaches 0.1-0.5mM. Continue the induction culture for 12-24h.)

4) After induction, the bacteria were collected by centrifugation, and the total protein, supernatant and precipitated components of the lysate of the bacteria were analyzed by appropriate methods(such as Coomassie brilliant blue stain method, Western-Blot method or enzyme activity analysis), and the expression status of the products(soluble or insoluble expression) was clearly expressed.
5) For large amounts of expression, 10mL of overnight culture can be transferred to 1L medium, and when the culture is OD600=0.4-0.8, IPTG with final concentration of 0.4mM is added, and the optimal conditions for expression of different proteins are different, and need to be optimized in the experiment.

Notes:

1. If the biochemical reagents produced by our company are not specially marked, they are basically non-sterile packaging. If they are used in cell experiments, please pre-treat them in advance.
2. Once it is prepared into a solution, please pack it separately and store it to avoid product failure caused by repeated freezing and thawing.
3. The product information is for reference only, if you have any questions, please call 400-968-6088 for consultation.
4. 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.
5. For your safety and health, please wear a lab coat and wear disposable gloves and a mask.