OrigamiB(DE3)plysS Competent Cells

Cat: C3720

Size: 10×100μL/20×100μL

Storage: Store at -70°C to avoid repeated freezing and thawing.

Product Parameters:

English name: OrigamiB(DE3)pLysS Competent cells

Product Components:

Components	Size	
OrigamiB(DE3)pLysS Competment Cells	10×100μL	20×100μL
pUC19 (0.1ng/μL)	5μL	5μL

Genotype:

F -ompT hsdS_B($r_B^-m_B^-$) gal dcm lacY1 ahpC (DE3) gor522::Tn10 trxB (Kan^R,Tet^R) pLysS Cam^R

Introduction:

The Origami B(DE3)pLysS strain carries the pLysS plasmid and is resistant to chloramphenicol. pLysS can express T7 lysozyme(T7 lysozyme can act on peptidoglycan on Escherichia coli cell wall to dissolve Escherichia coli, and can bind to T7 RNA polymerase to inhibit its transcriptional activity, thereby reducing the background expression level of target genes, but does not interfere with IPTG-induced expression), and is suitable for expressing toxic and non-toxic proteins. Origami B(DE3)pLysS strains express mutated thioredoxin reductase(trxB) and glutathione reductase(gor), which are key enzymes in the reduction pathway. Mutated to facilitate the formation of correctly folded proteins containing disulfide bonds, enhancing protein solubility. In addition, the chromosome of this strain integrates the λ phage DE3 region(DE3 region contains the T7 phage RNA polymerase), can express the T7 RNA polymerase and Escherichia coli RNA polymerase at the same time, can be used for the protein expression of pET series, pGEX, pMAL and other plasmints, has chloramphenicol, kanamycin and tetracycline resistance, can not be used for chloramphenicol, Kanamycin resistance plasmid expression. Origami B(DE3)pLysS competment cells were prepared by a special process and pUC19 plasmid was used to detect the conversion efficiency >10^7 cfu/μg DNA.

Protocols:

1. Plasmid transformation steps

1) The competment 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 SOC or LB medium;

6) The culture was placed in a shaking table at 37℃, 150-200rpm for 60min.
7) Take 50-100μL bacterial solution and apply it on LB plate containing resistance. After the liquid was drained, the plate was turned upside down and cultured at 37℃ for 12-16h.

(**Plate scribing separation method:** After the resuscitation culture, centrifuge at 12000rpm for 30s, discard the supernatant, leave about 100μL of liquid, gently blow the bacterial mass 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.
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 research 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.