W3110(DE3) Competent Cells

Cat: C3710
Size: 10×100μL/20×100μL
Storage: Store at -70°C to avoid repeated freezing and thawing.

Product Parameters:
English name: W3110(DE3) Competent cells

Genotype: F $^{-}\lambda^{-}$ rph-1INV (rrnD, rrnE)(lavUV5::T7 polymerase)(Cam^{R})

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

Introduction:

W3110(DE3) was derived from K-12 strain. The original expression box of T7 RNA polymerase (T7RNP) was integrated on the chromosome DNA of the bacteria. T7RNP was located under the promoter of lacUV5 and could express T7 RNA polymerase. Prokaryotic expression vectors such as pET, pGEX and pMAL can be used for the expression of exogenous recombinant proteins. W3110(DE3) competent cells were prepared by a special process. The conversion efficiency of pUC19 plasmid was greater than 1×10^{6} 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) 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 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 5 minutes, 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) When a large number of proteins are expressed, 10mL overnight culture can be transferred to 1L medium. When the culture reached OD600=0.4-0.8, IPTG with a final concentration of 0.4mM was added and induced at 37℃ for 2-4h or 16℃ overnight(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.