

DH10B-T1 Competent Cells

Cat: C3690

Size: 10×100μL/20×100μL

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

Product Parameters:

English name: DH10B-T1 Competent cells

Genotype: F⁻mcrA Δ(MR-HSDRms-MCRBC) φ80lacZ δM15δlacx74 recA1 endA1 araD139 δ(ara, leu)7697 galU galK⁻ λ rpsL nupG tonA

Introduction:

DH10B-T1 is an improved strain of DH10B. This strain is resistant to T1 and T5 phages. Deletion of nuclease(endA1) gene improved plasmid yield and quality; And mcrA genotype marker and mcrBC, mrr deletion made the strain suitable for cloning plasmid DNA containing methylcytosine and methyladenine. It is suitable for cloning and augmenting plasmids, constructing gene banks or generating cDNA libraries using plasmid derived vectors. The φ80lacZΔM15 marker provides α-complementation of β-galactosidase gene, and only IPTG and X-gal need to be added to perform blue-white spot screening experiments based on α-complementation principle. DH10B-T1 competent cells are prepared by a special process and can be used for chemical transformation of DNA. pUC19 plasmid was used to detect the conversion efficiency up to 10⁸ cfu/μg.

Protocols:

1. Take the competent cells and place them in the ice bath. If necessary, the freshly melted cell suspension can be divided into a sterile pre-cooled centrifuge tube and placed in the ice bath. (The recommended dosage for one conversion of competent cells is 50-100μL, which can be used in separate packs according to actual conditions. It should be noted that the volume of DNA used should not exceed one-tenth of the suspended fluid volume of the competent cells.

The following experiment takes 100μL competent cells as an example:

2. The target DNA was added to the competent cell suspension, the centrifuge tube was gently rotated to mix the contents, and the contents were left in an ice bath for 30min.
3. Place the centrifuge tube in a 42°C water bath for 60s, then quickly transfer the tube to the ice bath and allow the cells to cool for 2 minutes without shaking the centrifuge tube.
4. Add 500μL sterile SOC or LB medium(without antibiotics) to each centrifuge tube, mixed and placed at 150rpm at 37°C for 60min oscillating culture in a shaking table. The purpose was to express the related resistance marker genes on the plasmid and resuscitate the bacteria.
5. Under aseptic conditions, appropriate amount of bacterial solution was added to LB solid medium plate containing corresponding antibiotics, and the cells were evenly coated with sterile bacterial coater or glass beads. After the liquid in the plate was completely absorbed, the plate was inverted and cultured at 37°C for 12-16h. (The amount of coating can be adjusted

according to the specific experiment. The transformation plasmid is about 10ng, 90mm plate coated with 100 μ L, 55mm plate coated with 50 μ L; It is recommended that the transformed bacterial solution of the connected product be centrifuged and most of the supernatant be poured away, the remaining 200 μ L, and 100 μ l be used for coating.

6. Keep the remaining bacterial solution in the refrigerator at 4°C, and decide whether to leave or leave according to the colony growth on the plate.

Notes:

1. The competent cells should be kept at -70°C, and should not be frozen and thawed many times and placed for too long to avoid reducing the conversion efficiency of the competent cells.
2. When performing the conversion operation, it should be carried out according to the requirements of the corresponding temperature and aseptic conditions.
3. In order to prevent the conversion experiment from being unsuccessful, part of the connecting reaction liquid can be retained for re-conversion and the loss can be minimized.
4. If the biochemical reagents produced by our company are not specially marked, they are basically non-aseptic packaging. If they are 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 research 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.