DH5α-T1 Competent Cells

Cat: C1180
Size: 10×100μL
Storage: Store at -70℃ for 6 months to avoid repeated freezing and thawing. Not suitable for storage in liquid nitrogen.

Introduction:

DH5α-T1 is an improved strain of conventional DH5α. Deletion of nuclease(endA1) gene improves plasmid yield and quality; Recombinase defect type(recA1) reduces the homologous recombination probability of inserted fragments and ensures the stability of inserted DNA. Due to the presence of lacZΔM15 and the deletion of lacIq gene, it is not necessary to add IPTG, but only need to add X-gal to perform the blue-white spot screening experiment based on the principle of α-complementarity. The mutation of DH5α-T1 in the tonA region of the genome enables the strain to acquire resistance to T1 and T5 phage infection. DH5α-T1 competent cells are make by a special process, and the conversion efficiency of pUC19 plasmid was as high as 10^8 cfu/μg DNA.

Genotype: F- φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *rec*A1 *end*A1 *hsd*R17(rk-, mk+) *pho*A *sup*E44 *thi*-1 *gyr*A96 *rel*A1 *ton*A

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

Protocols: (The following operations are performed according to the standard of sterile conditions)

(Take ampicillin resistant pUC19 plasmid as an example)

1. The competent cells are melted in an ice bath. After the cells are just defrost, add 1-5μL of plasmid DNA containing 1-100ng into 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 at 42°C for 60s, do not shake.
4. Place in the ice bath for 2min without shaking.
5. Add 500μL SOC or LB medium at room temperature.
6. The culture was resuscitated in a 37°C shaker at 150-200rpm for 60min.
7. 50-100μL bacterial solution was applied on an ampicillin resistant LB plate. After the liquid was drained, the inverted plate was incubated at 37°C for 12-24h.

（Plate scribing separation method: After the resuscitation culture, centrifuge at 12000rpm for 30 seconds, discard the supernatant, leave about 100μL of liquid, gently blow the bacterial mass with 200μL suction head, take 10μL of suspended bacterial liquid and drop more 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 allows for larger monoclonal colonies.）

Notes:

1. The competent cells should be kept at -70℃, and cannot be frozen or thawed repeatedly, otherwise its conversion efficiency will be reduced.
2. During the experiment, aseptic operation should be strictly carried out to prevent contamination of other DNA or miscellaneous bacteria, so as to avoid influence on future screening and identification.
3. During the conversion, the conversion efficiency is proportional to the concentration of foreign DNA within a certain range, but when the amount of foreign DNA added is too large or too large, the conversion efficiency will be reduced. The volume of DNA during transformation should be less than one-tenth of the volume of competent cells.
4. Calculation of conversion rate: Conversion rate = total number of colonies produced/total amount of paving DNA.
5. In case the conversion experiment is unsuccessful, part of the junction product can be retained for re-conversion to minimize the loss.

Related Products:

I1020 IPTG solution(50mg/mL)
A1170 Ampicillin storage Solution(100mg/mL)
K1030 Kanamycin(100mg/mL)
L1015 LB solid medium(dry powder)
L1020 SOC Liquid medium(dry powder)
X1010 X-gal(20mg/mL)