dam-/dcm- Competent Cells

Cat: C1320
Size: 20×100μL
Storage: Store at -70°C for 6 months to avoid repeated freezing and thawing. Not suitable for storage in liquid nitrogen.

Introduction:

dam-/dcm- competent cells produced by our company are the receptive cells obtained by special processing of E. coli dam-/dcm- competent cells. The dam-/dcm- strain was derived from E. coli K12 strain, which was a dam and dcm methylase inactivated mutant strain, and was often used for de-dam and dcm methylation treatment of plasmid DNA to eliminate the effect of dam and dcm methylation on enzyme digestion. Deletion of endA1 gene was beneficial to improve the yield and quality of plasmid DNA. The inactivation mutation of the methylase may result in the mutation of the plasmid DNA in this strain, and the transformation experiment of the linker product is not recommended, only for plasmid transformation. The strain is also resistant to T1 phage infection. pUC19 plasmid detected conversion efficiency>×10^6 cfu/μg DNA.

Genotype: *ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22mcrA dcm-6 hisG4 rfbD1 R(zgb210::Tn10)* Tet^S *endA1 rspL136* (Str^R)*dam13::Tn9 (*Cam^R*) xylA-5 mtl-1 thi-1 mcrB1 hsdR2*

Strain Resistance: Resistant to chloramphenicol, streptomycin; Sensitive to ampicillin, kanamycin, spectacular and tetracycline.

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

1. The competent cells were placed in an ice bath to melt. After the cells were just melted, 1-5μL of plasmid DNA containing 1-100ng was added to the cells, dial the bottom of the tube with fingers, and gently mixed.
2. Let it sit in an ice bath for 30min.
3. Heat at 42°C for 60s without shaking.
4. Leave in the ice bath for 2min.
5. Add 500μL room temperature SOC or LB medium.
6. The culture was placed in a shaker at 37°C and resuscitated by shock at 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°C for 12-18h.

(Plate scribing separation method: After the recovery 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 body dripping on the plate. This method can obtain a larger monoclonal colony. This method is mainly suitable for plasmid

transformation, and the conversion of link products is best by coating.)

(Rapid plasmid transformation steps: Shorten the time of step 2 to 5min. For ampicillin resistant plasmids, after the completion of step 4, they can be directly coated or marked on the ampicillin resistant LB plate. For other resistant plasmids, 60min of resuscitation was required.)

Notes:

1. The receptive 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)