



Rosetta (DE3) Chemically Competent Cell

产品货号： BDC201

产品规格： 100μl/支

基因型： *FompT hsdS_B(r_Bm_B⁻) gal dcm(DE3) pRARE(argU, argW, ileX, glyT, leuW, proL) (Cam^R)*

产品介绍：

Rosetta(DE3)菌株具有氯霉素抗性，补充大肠杆菌缺乏的6种稀有密码子(AUA, AGG, AGA, CUA, CCC, GGA)对应的tRNA，提高外源基因，尤其是真核基因在原核系统中的表达水平，该菌株染色体整合了λ噬菌体DE3区(DE3区含有T7噬菌体RNA聚合酶)，同时表达T7 RNA聚合酶和大肠杆菌RNA聚合酶，可用于pET系列，pGEX，pMAL等质粒的蛋白表达。Rosetta(DE3)感受态细胞由特殊工艺制作，pUC19质粒检测转化效率高达10⁸ cfu/μg DNA。

操作方法：

1. Rosetta(DE3)感受态细胞从-80℃拿出，迅速插入冰中，5分钟后待菌块融化，加入目的DNA（质粒或连接产物）并用手拨打EP管底轻轻混匀(避免用枪吸打)，冰中静置25分钟。
2. 42℃水浴热激45秒，迅速放回冰上并静置2分钟，晃动会降低转化效率。
3. 向离心管中加入700μl不含抗生素的无菌培养基(2YT或LB)，混匀后37℃，200rpm复苏60分钟。
4. 5000rpm离心一分钟收菌，留取100μl左右上清轻轻吹打重悬菌块并涂布到含相应抗生素的2YT或LB培养基上。
5. 将平板倒置放于37℃培养箱过夜培养。

Sample Induction Protocol (for reference only)

1. Inoculate a single colony from a freshly streaked plate into 5 ml of LB medium containing the appropriate antibiotic for the plasmid and host strain.
2. Incubate with shaking at 200 rpm at 37℃ overnight.
3. Inoculate 50 ml of LB medium containing the appropriate antibiotic with 0.5 ml of the overnight culture prepared in step 2 (use the 500 ml triangular flask as the container would be better).
4. Incubate with shaking at 150 rpm at 37℃ until the OD 600 reaches 0.5-0.8.
5. (Optional) Pipet 1ml of the cultures into clean microcentrifuge tubes and place the tubes on ice until needed for gel analysis or storage at -20℃. These will serve as the non-induced control samples.
6. Add IPTG to a final concentration of 1 mM. Optimal time for induction of the target protein may vary from 2-16 hours, depending on the protein.
7. Incubate with shaking at 120 rpm at 37℃ for 3-4 hours. To determine the optimal time for induction of the target protein, it is recommended that a time course experiment be performed varying the induction from 2-16 hours.
8. Place the culture on ice for 10 minutes. Harvest cells by centrifugation at 5,000 ×g for 10 minutes at 4℃.
9. Remove the supernatant and store the cell pellet at -20℃ (storage at lower temperatures is also acceptable).

IPTG

Prepare a 1 M solution of IPTG (Isopropyl-β-D-thiogalactoside; Isopropyl-β-D-thiogalactopyranoside) by dissolving 2.38 g of IPTG in dd water and adjust the final volume to 10 ml. Filter sterilize before use.

氯霉素



Chloramphenicol 34 mg/ml in ethanol. Store at -20°C. Use at 34 µg/ml.

注意事项：

1. 感受态细胞最好在冰中缓慢融化，插入冰中 8 分钟内加入目标 DNA，不可在冰中放置时间过长，长时间存放会降低转化效率。
2. 混入质粒时应轻柔操作。
3. 转化高浓度的质粒可相应减少最终用于涂板的菌量。
4. 诱导时，IPTG 浓度可选(0.1-2mM 均可)。
5. 为获得需要量的蛋白，最佳诱导时间，温度，IPTG 浓度需实验者优化。

保存条件：

-80°C 保存 6 个月。