Rosetta(DE3) 感受态细胞

Rosetta(DE3) Chemically Competent Cell

保存条件： -80℃

产品规格:

Rosetta(DE3)	10×100μl
pUC19（control vector）	10pg/μl；10μl

基 因 型

F^-ompT $hsdS_B(r_B^-m_B^-)$gal dcm(DE3) pRARE(argU，argW，ileX，glyT，leuW，proL)(Cam^R)

简 要 说 明

Rosetta(DE3) 感受态细胞具有氯霉素抗性，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，经 pUC19 质粒检测转化效率高达 10^8cfu/μg。

操 作 说 明

1. 取 100μl 冰上融化的 Rosetta(DE3)感受态细胞，加入目的质粒并轻轻混匀，冰上静置 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。

注 意 事 项

1. 感受态细胞最好在冰上融化。
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。

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 x g for 10

1. 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) bydissolving 2.38 g of IPTG in dd water and adjust the final volume to 10 ml. Filter sterilize before use.