

Rosetta-gami 2(DE3)

Rosetta-gami 2(DE3) Chemically Competent Cell 说明书

产品货号: ML-G27019

保存条件: -80℃

产品规格: 10×100μl 50×100μl

产品介绍

基 因 型

D(ara-leu)7697 DlacX74 DphoA Pvull phoR araD139 ahpC galE galK rpsL
(DE3) F' [lac+ lacIq pro] gor522::Tn10 trxB pRARE2 (CamR, StrR, TetR)

简 要 说 明

Rosetta-gami 2(DE3) 菌株集合了 Rosetta 2 和 Origami 2 两种菌株的优点:

pRARE2 赋予其 Rosetta2 菌株的优点——补充大肠杆菌缺乏的 7 种稀有密码子(AUA, AGG, AGA, CUA, CCC, GGA 和 CGG)对应的 tRNA，提高外源基因的表达水平。 gor522::Tn10 trxB 赋予其 Origami2 菌株的优点——突变的硫氧还蛋白还原酶(thioredoxin reductase) (trxB)和谷胱甘肽还原酶(glutathione reductase) (gor)基因，它们是还原途径的两个关键酶，其突变有利于高效形成正确折叠的含有二硫键的蛋白，增强蛋白的可溶性；同时该菌株不具有卡那霉素抗性，可用于具有卡那霉素抗性质粒的蛋白表达。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)适合 T7 启动子诱导的蛋白表达。Rosetta-gamiB(DE3)菌株具有氯霉素，链霉素，四环素抗性，由特殊工艺制作，经 pUC19 质粒检测转化效率高达 108 cfu/ μ g DNA。

操 作 说 明

1. Rosetta-gami 2(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，加入目的质粒并用手拨打 EP 管底混匀，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 700 μ l 不含抗生素的无菌培养基（2YT 或 LB），混匀后 37℃，200 rpm 复苏 60 分钟。
- 4.5000 rpm 离心一分钟收菌，留取 100 μ l 左右上清轻轻吹打重悬菌块并涂布到含所选质粒筛选抗生素的 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（0.1-10 mM 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 3ml of LB medium containing the appropriate antibiotic for the plasmid and host strain.
2. Incubate with shaking at 200 rpm at 37°C 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°C until the OD 600 reaches 0.5-0.8. (0.6 recommended; about 2.5h).
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°C. 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°C for 2-4 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

minutes at 4 °C.

9. Remove the supernatant and store the cell pellet at -20 °C (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.