



Rosetta-gami 2(DE3) Chemically Competent Cell

产品货号： BDC213-1

产品规格： 100 μ l/支

基因型：

*D(ara-leu)7697 DlacX74 DphoA PvuII phoR araD139 ahpC galE galK rpsL (DE3) F[*lac+* *lacIq* *pro*] gor522::Tn10 trxB pRARE2 (*Cam^r*, *Str^r*, *Tet^r*)*

产品介绍：

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-gami 2(DE3)菌株具有氯霉素，链霉素，四环素抗性，由特殊工艺制作，转化效率高达 10^8 cfu/ μ g DNA。

操作方法：

1. Rosetta-gami 2(DE3)菌株感受态细胞从-80 $^{\circ}$ C拿出，迅速插入冰中，5 分钟后待菌块融化，加入目的 DNA 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 $^{\circ}$ C 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 $^{\circ}$ C，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 $^{\circ}$ C 培养箱过夜培养。

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 $^{\circ}$ 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 $^{\circ}$ 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 $^{\circ}$ 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 $^{\circ}$ 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 $\times$ g for 10 minutes at 4 $^{\circ}$ 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-thiogalactopyran-oxide) by dissolving 2.38 g of IPTG in dd water and adjust the final volume to 10 ml. Filter sterilize before use.

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（0.1-2 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。

保存条件：

-80°C 保存 6 个月。