

OrigamiB(DE3)感受态细胞

OrigamiB(DE3) Chemically Competent Cell 说明书

产品货号: ML-G2027

保存条件: -80℃

产品规格: 10×100μl 50×100μl

产品介绍

基 因 型

F-ompT hsdSB(rB-mB-) gal dcm lacY1 ahpC (DE3) gor522::Tn10 trxB
(KanR, TetR)

简 要 说 明

OrigamiB(DE3)感受态细胞包含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, 表达主要还原途径的两个关键酶。有利于形成正确折叠的含有二硫键的蛋白, 增强蛋白的可溶性。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), 可同时表达 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, 具有卡那霉素和四环素抗性, 不能用于具有卡那霉素抗性质粒的表达。MLBio High5™ 系列 OrigamiB (DE3) 感受态细胞由特殊工艺制作, 经 pUC19 质粒检测转化效率达 $108\text{cfu}/\mu\text{g}$ 。

操作说明

1. 取 $100\mu\text{l}$ 冰上融化的 OrigamiB(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°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 $700\mu\text{l}$ 不含抗生素的无菌培养基(2YT 或 LB), 混匀后 37°C , 200rpm 复苏 60 分钟。
4. 5000rpm 离心一分钟收菌, 留取 $100\mu\text{l}$ 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°C 培养箱过夜培养。

注意事项

1. MLBio 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。
6. 具有卡那霉素抗性，不能用于具有卡那霉素抗性质粒的表达。

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.