

B834(DE3) Chemically Competent Cell 产品说明书

● 产品规格 (CAT# : EC2040)

B834(DE3) Competent Cell	100μl /支
pUC19 (control vector, 10pg/μl)	10μl
保存条件 (保质期):	-80°C (6个月)

● 基因型

F- *ompT hsdS_B(r_B- m_B-) gal dcm met* (DE3)

● 产品说明

B834(DE3)是 BL21(DE3)菌株的亲本菌株 (BL21(DE3)为 B834(DE3)的衍生菌株), 广泛用于蛋白的原核表达实验。B834(DE3)是甲硫氨酸营养缺陷型菌株, 可用于 ³⁵S-methionine 标记蛋白的实验或通过硒代甲硫氨酸用于晶体学研究。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), 该区整合于大肠杆菌的染色体上, 可同时表达 T7 RNA 聚合酶和大肠杆菌 RNA 聚合酶, 用于 pET 系列, pGEX, pMAL 等质粒的蛋白表达。B834(DE3)感受态细胞由特殊工艺制作, pUC19 质粒检测转化效率 > 1 × 10⁸ cfu/μg DNA。

● 操作方法

1. B834(DE3)感受态细胞从-80°C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 管底轻轻混匀(避免用枪吸打), 冰中静置 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 μl 不含抗生素的无菌培养基 (2YT 或 LB), 混匀后 37°C, 200 rpm 复苏 60 分钟。
4. 5000 rpm 离心一分钟收菌, 留取 100 μl 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。

● 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×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 配制:

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.

● 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. 转化高浓度的质粒可相应减少最终用于涂板的菌量。
3. 诱导时，IPTG 浓度可选 (0.1-2 mM 均可)。
4. 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。