B834(DE3)感受态细胞

B834(DE3)ChemicallyCompetent Cell 说明书

产品货号： **ML-G2066**

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-) galdcm met(DE3)

简 要 说 明

MLBioB834 是 BL21 菌株的父系菌株。该系列宿主菌是蛋氨酸缺陷型，可以

用来高水平，高特异性 的使用 35S-蛋氨酸和硒代蛋氨酸标记目的蛋白，进行蛋白质晶体结构研究。B834 宿主菌也是 lon 和 ompT 蛋白酶缺陷型菌株。DE3 是溶源性的λDE3, 所以带有 T7RNA 聚合酶的染色体拷贝。该菌株适用于 pET 系列载体，及其他 T7 启动子系列载体。

操 作 说 明

1.取 100μl 冰上融化的 B834（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. 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（0.1-40mM 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。

SampleInduction Protocol (for reference only)

Inoculatea single colony from a freshly streaked plate into 5 ml of LB medium containingthe appropriate antibiotic for theplasmid andhost strain. 2. Incubate with shakingat 200 rpm at 37℃ overnight. 3. Inoculate50 ml of LB medium containing the appropriateantibiotic with 0.5ml of the overnight culture prepared in step 2(usethe 500 ml triangular flask as thecontainer would bebetter). 4. Incubate with shakingat 150 rpm at 37℃ until the OD 600 reaches 0.5-0.8. 5. (Optional)Pipet1ml 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 thenon-induced control samples. 6. AddIPTG to a final concentration of 1 mM. Optimal time for induction of thetarget protein may vary from 2-16 hours, depending on the protein. 7. Incubatewithshakingat120rpmat37℃ for3-4hours.Todeterminetheoptimaltimeforinduction of the target protein, it is recommended that a time course experiment beperformed varying the induction from 2-16 hours. 8. Place theculture on ice for 10 minutes. Harvest cells by centrifugation at 5,000 x g for 10 minutes at 4℃. 9. Remove the supernatant andstore 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) by dissolving 2.38 g of IPTG in dd water andadjust the final volume to 10 ml. Filter sterilize before use.