



BL21-CodonPlus (DE3)-RIPL Chemically Competent Cell

产品货号： BDC107-1

产品规格： 100μl/支

基因型： *F-ompT hsdS(rB-mB-) dcm+ TetR gal λ(DE3) endA Hte [argU proL CamR] [argU ileY leuW Strep/SpecR]*

产品介绍：

BL21-CodonPlus(DE3)-RIPL 菌株来源于 Stratagene 公司的 BL21-Gold 菌株，缺少 Lon 蛋白酶和 OmpT 蛋白酶，从而减少对重组蛋白的降解，补充大肠杆菌缺乏的 4 种稀有密码子 (AGA, AUA, CCC, CUA) 对应的 tRNA (*argU*, *ileY*, *proL*, *leuW*)，提高外源基因，尤其是富含 AT-或 GC-的真核基因在原核系统中的表达水平。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，同时具有四环素，氯霉素，链霉素，壮观霉素抗性。BL21- CodonPlus(DE3)-RIPL 感受态细胞由特殊工艺制作，转化效率高达 10^8 cfu/μg DNA。

操作方法：

1. BL21-CodonPlus(DE3)-RIPL 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 DNA (质粒或连接产物) 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℃ 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。

Sample Induction Protocol (for reference only)

1. Inoculate a single colony from a freshly streaked plate into 5 ml of LB medium containing the appropriate antibiotic for the plasmid and host strain.
2. Incubate with shaking at 200 rpm at 37℃ 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℃ until the OD 600 reaches 0.5-0.8.
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℃. 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℃ for 3-4 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℃.
9. Remove the supernatant and store 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 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(0.1-2mM 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。

保存条件：

-80℃保存 6 个月。