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ZOMANBIO

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ArcticExpress(DE3) RP 感受态细胞

ArcticExpress(DE3) RP Chemically Competent Cell

Cat.NO. ZC1219

目录编号	产品名称	包装单位
☐ ZC1219-1	ArcticExpress(DE3) RP 感受态细胞	10×100μl
☐ ZC1219-2	ArcticExpress(DE3) RP 感受态细胞	20×100μl

备注：以上包装均含有 Compcell Control Plasmid pUC19(0.1ng/μl) 5μl（质量控制用）。

储存：-70℃ 保存六个月。

产品介绍：

本公司生产的 ArcticExpress(DE3) RP 感受态细胞是采用特殊工艺处理得到的感受态细胞，可用于 DNA 的化学转化，具有庆大霉素抗性。使用 pUC19 质粒检测，转化效率高达 10^7 cfu/μg DNA 以上。

产品特点：

ArcticExpress RP Competent Cells are engineered to address the common bacterial gene expression hurdle of protein insolubility. These cells are derived from the high-performance Stratagene BL21-Gold competent cells, enabling efficient high-level expression of heterologous proteins in Escherichia coli.

Low growth temperatures enhance protein folding and solubility.

Feature chaperonins Cpn60 and Cpn10 from psychrophilic bacterium Oleispira Antarctica.

Correct for codon bias and enhance solubility at the same time.

操作步骤：

以下操作均按无菌条件的标准进行：

- **转化:**取感受态细胞置于冰浴中(解冻 1-2 分钟),加入目的 DNA,轻轻混匀,在冰浴中放置 30 分钟。
  注意:所使用 DNA 体积不要超过感受态细胞悬液体积的 1/10,100μl 感受态细胞能够被 1ng 超螺旋质粒 DNA 所饱和。
- **热激:**将离心管置于 42℃水浴中放置 60-90 秒，然后快速将管转移到冰浴中，使细胞冷却 2-3 分钟,该过程不要摇动离心管。
- **复苏:**向每个离心管中加入 500μl 无菌的 SOC 或 LB 培养基（不含抗生素），混匀后置于 37℃ 180rpm 摇床振荡培养 45-60 分钟，目的是使质粒上相关的抗性标记基因表达，使菌体复苏。
- **涂板:**根据实验要求（质粒，重组连接产物转化），吸取适量体积已转化的感受态细胞加到含相应抗生素的 SOC 或 LB 固体琼脂培养基上，将细胞均匀涂开。将平板置于室温直至液体被吸收，倒置平板，37℃培养 12-16 小时。

提示：

· 刚刚化冻的细胞，转化效率最高。化冻后感受态细胞冰浴条件下，半小时内活性无明显变化，因此，同时转化多支感受态细胞时尽量半小时内加完目的 DNA。

· 感受态细胞应保存在 -70℃，请避免反复冻融，以免降低感受态细胞的转化效率。

· 进行转化操作时，请在无菌条件下，根据相应温度要求进行实验。

· 避免用移液枪吹吸，整个过程要轻柔，尽量低温操作。

· 为防止转化实验不成功，可以保留部分连接反应液，以重新转化，将损失降到最低。

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 200rpm at 37℃ overnight.

3. Inoculate 50ml of LB medium containing the appropriate antibiotic with 0.5ml of the overnight culture prepared in step 2(use the 500ml triangular flask as the container would be better).

4. Incubate with shaking at 150rpm at 37℃ 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℃ . These will serve as the non-induced control samples.

6. Add IPTG to a final concentration of 1mM. Optimal time for induction of the target protein may vary from 2-16hours, depending on the protein.

7. Incubate with shaking at 120rpm at 37℃ for 2-4hours. 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 2-16hours.

8. Place the culture on ice for 10minutes. Harvest cells by centrifugation at 5,000×g for 10minutes at 4℃ .

9. Remove the supernatant and store the cell pellet at -20℃ (storage at lower temperatures is also accep- table).

IPTG 配制：

Prepare a 1M solution of IPTG (Isopropyl-β-D-thiogalactoside; Isopropyl-β-D-thiogalactopyranoside) by dissolving 2.38g of IPTG in dd water and adjust the final volume to 10ml. Filter sterilize before use.