



上海源叶生物科技有限公司
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Origami2(DE3) 感受态细胞

Origami2(DE3) Chemically Competent Cell

产品货号: S32866

保存条件: -80°C

产品规格: 100ul*10、100ul*50

基 因 型:

$\Delta(\text{ara-leu})7697 \Delta\text{lacX74} \Delta\text{phoA PvuII phoR araD139 ahpC}$

$\text{galE galK rpsL F'[\text{lac}^+ \text{lacIq pro}] (DE3)}$

$\text{gor522::Tn10trxB (StrR, TeR)}$

简 要 说 明:

Origami 2 系列菌株是 K-12 菌株衍生而来，在 thioredoxin reductase (trxB) 和 glutathione reductase (gor) 基因上同时含有突变，这使得该菌株能够更加高效的在细胞质内生成二硫键，有助于含二硫键蛋白的活性蛋白形成。与 Origami 系列菌株相比，Origami 2 系列菌株是卡那敏感的，这使得该菌株能够适用于大多数的蛋白表达质粒。Gor 基因突变使得该菌株和原来的 Origami(DE3) 菌株一样具有四环素抗性。DE3 是溶源性的 λ DE3，所以在 lacUV5 启动子下携带有 T7 RNA 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。Origami2 (DE3) 同时还是亮氨酸生长缺陷型宿主菌，可用于相关的克隆以及蛋白表达，在 IPTG 的诱导下可进行蛋白表达。AngYuBioHigh5™ 系列 Origami2 (DE3) 感受



态细胞由特殊工艺制作，经 pUC19质粒检测转化效率达108cfu/μg。

操作说明

- 1.取100μl 冰上融化的 Origami2(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2YT 或 LB 培养基，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.AngYuBio 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。

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°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.
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 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 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).



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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

