XL10-Gold Chemically Competent Cell 产品说明书

● 产品规格（CAT#：DL1050）

XL10-Gold Competent Cell	100μl /支
pUC19 (control vector, 10pg/μl)	10μl
保存条件（保质期）：	-80℃（6 个月）

● 基因型

Tet^R Δ(*mcr*A)183 Δ(*mcr*CB-*hsd*SMR-*mrr*)173 *end*A1 *sup*E44 *thi*-1 *rec*A1 *gyr*A96 *rel*A1 *lac* Hte [F′ *pro*AB *lacI*^{q}ZΔM15 *Tn*10 (Tet^R) Amy Cam^R]

● 产品说明

XL10-Gold 是目前转化效率最高的感受态细胞，由 Stratagene 开发的特异性用于大质粒或珍贵连接产物转化或构建文库的超级感受态细胞。XL10-Gold 菌株为 Hte (high transformation efficiency)基因型，Hte 是 Stratagene 开发的特异性提高感受态转化效率及大质粒转化能力的宿主菌基因型，已成功应用于 40 kd 质粒的构建。[Δ(*mcr*A)183 *Δ(mcr*CB-*hsd*SMR-*mrr)*173]赋予 XL10-Gold 缺失几乎所有已知的限制酶切系统；同时缺失核酸内切酶 (endA)，提高了质粒 DNA 的产量和质量；重组酶缺陷型(recA)减少插入片段的同源重组概率，保证了插入 DNA 的稳定性；Tet^R，Cam^R 赋予菌株四环素和氯霉素抗性；*lacI*^{q}ZΔM15 的存在使 XL10-Gold 可用于蓝、白斑筛选。XL10-Gold 感受态细胞经特殊工艺制作，pUC19 质粒检测转化效率>2×10^9 cfu/μg DNA。

● 常规操作方法

1. XL10-Gold 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 35 秒（非常重要——Efficiency decreases sharply when cells are heat-pulsed for <30 seconds or for >40 seconds.），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 1 分钟收菌，留取 100μl 左右上清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。

● Stratagene standard protocol

1. Pre-chill a 14-ml BD Falcon polypropylene round-bottom tubes on ice. Preheat NZY+ broth to 42℃.
2. Thaw the cells on ice. When thawed, gently mix and aliquot 100 µl of cells into the pre-chilled tubes.
3. Add 4 µl of the β-ME (β 巯基乙醇) to the aliquot of cells.
4. Swirl the tubes gently. Incubate the cells on ice for 10 minutes, swirling gently every 2 minutes.
5. Add 0.1-50 ng of the experimental DNA (or 2 µl of a ligation mixture) to the aliquot of cells.
6. Swirl the tubes gently, then incubate the tubes on ice for 30 minutes.
7. Heat-pulse the tubes in a 42℃ water bath for 30 seconds. The duration of the heat pulse is critical.
8. Incubate the tubes on ice for 2 minutes.
9. Add 0.9 ml of preheated (42℃) NZY+ broth and incubate the tubes at 37℃ for 1 hour with shaking at 225-250 rpm.
10. Plate ≤200 µl of the transformation mixture on LB agar plates containing the appropriate antibiotic (and containing IPTG and X-gal if color screening is desired).
11. Incubate the plates at 37℃ overnight. If performing blue-white color screening, incubate the plates at 37℃ for at least 17 hours to allow color development (color can be enhanced by subsequent incubation of the plates for 2 hours at 4℃).

● Preparation of Media and Reagents

NZY+ Broth (per Liter)

10 g of NZ amine (Casein hydrolysate)
5 g of Yeast extract
5 g of NaCl
Add deionized H_2O to a final volume of 1 liter
Adjust to pH 7.5 using NaOH and then autoclave
Add the following filer-sterilized supplements prior to use:
12.5 ml of 1 M $MgCl_2$
12.5 ml of 1 M $MgSO_4$
20 ml of 20% (w/v) glucose (or 10 ml of 2 M glucose)

LB Agar (per Liter)

10 g of NaCl
10 g of Tryptone
5 g of Yeast extract
20 g of Agar
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH and then autoclave
Pour into petri dishes (~25 ml/100-mm plate)

● 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. 混入目的 DNA 时应轻柔操作。
3. 转化高浓度的质粒或高效率的连接产物可相应减少最终用于涂板的菌量。
4. XL10-Gold 菌株对 <40 µg/ml 氯霉素有抗性，但对 100 µg/ml 氯霉素敏感。
5. XL10-Gold 感受态细胞采用常规转化方法，转化效率可达 2×10^9 cfu/µg DNA；如果有更高要求，可尝试 Stratagene 公司推荐的标准 protocol。