TaKaRa

# *E. coli* HST04 *dam*$^{-}$/*dcm*$^{-}$ Competent Cells

Cat.# 9129

Guide

## I. Contents

| | |
|---|---|
| *E. coli* HST04 *dam*⁻ / *dcm*⁻ Competent Cells | 100 μl × 10 |
| pUC19 plasmid （0.1 ng/ μl） | 10 μl |
| SOC media＊ | 1 ml × 10 |

＊：SOC media components：2 %Tryptone

| | |
|---|---|
| 0.5 % | Yeast extract |
| 10 mM | NaCl |
| 2.5 mM | KCl |
| 10 mM | $MgSO_4$ |
| 10 mM | $MgCl_2$ |
| 20 mM | Glucose |

## II. Features and Uses

*E. coli* HST04 *dam-/dcm*-competent cells are prepared by Hanahan's method modified by Takara Bio to achieve an extremely high transformation efficiency of >1 x $10^6$ cfu/μg when 100 μl of the cells are transformed with 1 ng pUC19.
*E. coli* HST04 *dam-/dcm-* lack the genetic factors *dam* and *dcm* found in wild type *E. coli* that are necessary for DNA methylation. Plasmids prepared using this product can be cut by restriction enzymes which are normally blocked by *dam* or *dcm* methylation.
A *dam*/*recA* double mutation is lethal, so the parent strain is *recA*+. Therefore, it follows that transformation of extracellular DNA with repeat sequences can result in recombination by *recA*. Therefore, this product is not suitable for routine DNA cloning other than preparation of unmethylated plasmids as mentioned above.

## III. Protocol (Transformation with a Plasmid Vector)

（1） Thaw *E. coli* HST04 *dam-/dcm-* Competent Cells on ice.
（2） After thawing, mix the cells gently to uniformity, and transfer 100 μl of competent cells into a 14 ml round-bottom tube (Falcon tube). Do not vortex to mix the cells.
（3） Add no more than 10 ng of DNA for transformation.
（4） Incubate tubes on ice for 30 minutes.
（5） Heat shock the cells for exactly 45 seconds at 42 ℃ .
（6） Place tubes on ice for 1 ～ 2minutes.
（7） Add SOC medium to a final volume of 1 ml. SOC medium should be warmed to 37℃ prior to use.
（8） Incubate at 37℃ for 1 hour with shaking at 160 ～ 225rpm.
（9） Plate an appropriate amount of culture.＊
（10） Place plates in a 37℃ incubator and grow overnight.

＊：Plate no more than 100μl for a 9 cm diameter plate. If necessary, dilute the culture with the same medium as used in step (7).

## IV. Please read before proceeding:

1. Place a tube of competent cells in a dry ice/EtOH bath immediately upon removal from the -80℃ freezer. Keep the cells in the bath until you are ready to proceed.
2. You may use 1.5 ml microcentrifuge tubes instead of 14 ml round bottom tubes (BD company Code: 352059 or 352057, etc.) for transformation, but it may reduce efficiency.
3. For 100 $\mu$ l of competent cells, use no more than 10 ng of high purity DNA or transformation efficiency might decrease.
4. If you change the quantity of competent cells, or type of tubes used, it might be necessary to reevaluate the conditions. For example, when using 1.5 ml microcentrifuge tubes, heat shock for 60 seconds at 42℃ rather than 45 seconds.
5. L-Broth or $\psi$-broth can be used instead of SOC medium, but efficiency may be reduced.
   - L-broth： 10 g Bacto tryptone, 5 g Bacto yeast extract, 5 g NaCl, pH to 7.5 with 1M NaOH, bring to a final volume of 1 L, and autoclave.
   - $\psi$ b-broth： 5 g Bacto yeast extract, 20g Bacto tryptone, 5 g $MaSO4{\bullet}7H_2O$, pH to 7.5 with 1M KOH, bring to a final volume of 1 L and autoclave.
6. L-plates: 10 g Bacto tryptone, 5 g Bacto yeast extract, 5 g NaCl, pH to 7.5 with 1M NaOH, add agar to be 1.5%, bring to a final volume of 1 L and autoclave.
7. When using X-Gal:
   - Add 20 mg /ml X-Gal (dissolved in dimethylformamide) at a ratio of 200 $\mu$ l/ 100 ml agar media.
8. Once competent cells have been thawed, refreezing for storage is not recommended. If this is unavoidable, flash freeze the cells on dry ice/ethanol and store them promptly at -80℃ . However, the transformation efficiency will be lowered by at least one order of magnitude.

## V. Quality

Efficiency of transformation

1 ng of pUC 19 plasmid was transformed according to the protocol in section III, and transformants were selected on an L-agar plate containing ampicillin. Then, the resulting transformation efficiency was $1 \times 10^8$ colonies / $\mu$g•pUC 19 plasmid.

## VI. Genotype

*E. coli* HST04 *dam*$^-$ / *dcm*$^-$：
F $^-$, *ara,* Δ(*lac-proAB*) [Φ80d *lacZ* ΔM15], *rps*L(*str*), *thi*, Δ(*mrr-hsdRMS-mcrBC*), Δ*mcrA*, *dam*, *dcm*

## VII. Cell density

$1 \sim 2 \times 10^9$ bacteria/ml

## VIII. Storage

− 80 ℃

Warning：Store at -80℃ or lower. If the storage temperature is not maintained consistently, the transformation efficiency may be reduced. You may determine the transformation efficiency of stored cells by using the included pUC19 control. Do not store in liquid nitrogen.

## IX. References


1) Hanahan,D. (1983) *J.Mol.Biol*., **166**, 557.
2) Messsing,J. (1985) *Gene* **33**,103.


## X. Related Products

pUC118 DNA（Cat.#: 3318）
pUC119 DNA（Cat.#: 3319）
Endonuclease cut pUC118 DNA (BAP treated)（Cat.#: 3320 ～ 3324）

## XI. Notes

- Our product is intended for research use only. Do not to use this product for human or animal medicine, or clinical diagnostics. Please do not use this product in foods, cosmetic items, or house supplies.
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