Cat. # 9128

For Research Use

TaKaRa

# *E. coli* HST08 Premium Competent Cells

Product Manual

v202007Da

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## I. Description

*E. coli* HST08 Premium Competent Cells are prepared by Hanahan's method modified by Takara Bio to achieve an extremely high transformation efficiency. In addition, *E. coli* HST08 Premium Cells lack the genes necessary for cutting foreign methylated DNA: *mrr*, *hsdRMS*, *mcrBC*, and *mcrA*. These properties make these competent cells useful in a wide range of applications such as methylated DNA cloning, genetic library construction, and routine subcloning. Even when working with very large plasmids, transformation efficiency and colony growth rates remain very high*. DNA cloning and genetic library construction with DNA inserts larger than 10 kb is possible when these cells are combined with the TaKaRa DNA Ligation Kit Long (Cat. #6024).

＊ Compared with other competent cells of the same genotype.

For transformation of pUC based plasmids, selection of recombinants may be simplified by blue-white screening using $\alpha$-complementarity to $\beta$-galactosidase of the competent cell on the X-Gal containing media.

X-Gal：5-Bromo-4-Chloro-3-Indolyl-$\beta$-D-Galactoside

## II. Components

| | | |
|---|---|---|
| *E. coli* HST08 Premium Competent Cells | 100 µl | x 10 |
| pUC19 plasmid (0.1 ng/ µl) | 10 µl | |
| SOC Medium＊ | 1 ml | x 10 |

＊ SOC Medium：

| | |
|---|---|
| 2% | Tryptone |
| 0.5% | Yeast extract |
| 10 mM | NaCl |
| 2.5 mM | KCl |
| 10 mM | $MgSO_4$ |
| 10 mM | $MgCl_2$ |
| 20 mM | Glucose |

## III. Storage

-80℃

**Note:** 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.

## IV. Protocol

Transformation with a Plasmid Vector

(1) Thaw *E. coli* HST08 Premium 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 min.
(5) Heat shock the cells for exactly 45 sec at 42℃.
(6) Place tubes on ice for 1 - 2 min.
(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 - 225 rpm.
(9) Plate an appropriate amount of culture.*
(10) Place plates in a 37℃ incubator and grow overnight.

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

**【 Read these before use 】**

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 (CORNING Code: 352059 or 352057, etc.) for transformation, but it may reduce efficiency.
3. For 100 μl 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 sec at 42℃ rather than 45 sec.
5. L-Broth or ψ-broth can be used instead of SOC Medium, but efficiency may be reduced.

   L-broth:

   | Ingredient | per liter water |
   |---|---|
   | Tryptone | 10 g |
   | Yeast extract | 5 g |
   | NaCl | 5 g |

   Adjust to around pH 7.5 with 1N NaOH, and autoclave.

   ψb-broth:

   | Ingredient | per liter water |
   |---|---|
   | Tryptone | 20 g |
   | Yeast extract | 5 g |
   | $MgSO_4$, $7H_2O$ | 5 g |

   Adjust to around pH 7.5 with 1N KOH and autoclave.

   L-plates:

   | Ingredient | per liter water |
   |---|---|
   | Tryptone | 10 g |
   | Yeast extract | 5 g |
   | NaCl | 5 g |

   Adjust to around pH 7.5 with 1N NaOH and add agar to 1.5%, then autoclave.

6. When using X-Gal:
   Add 20 mg /ml X-Gal (dissolved in dimethylformamide) at 200 - 300 μl/100 ml agar media.
7. 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

1 Efficiency of transformation
1 ng of pUC19 plasmid was transformed according to the protocol in section IV and transformants were selected on an L-plate containing ampicillin. The resulting transformation efficiency was > 1 x $10^8$ colonies/$\mu$g・pUC19 plasmid.
2 Confirmation of $\beta$-galactosidase $\alpha$-complementation.
When transformed with a pUC19 plasmid, blue colonies appeared on an L-agar plate containing 100 $\mu$g/ml ampicillin and 60 $\mu$g/ml X-Gal.

## VI. Genotype

*E. coli* HST08 Premium：
$F^-$, *endA1*, *supE44*, *thi*-1, *recA*1, *relA*1, *gyrA*96, *phoA*, Φ80d *lacZ*ΔM15, Δ(*lacZYA - argF*)*U169*, Δ(*mrr - hsdRMS - mcrBC*), Δ*mcrA*, $\lambda^-$

## VII. Cell density

1 - 2 x $10^9$ bacteria/ml

## VIII. References

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

## IX. Related Products

*E. coli* HST08 Premium Electro-Cells (Cat. #9028)
TaKaRa DNA Ligation Kit LONG (Cat. #6024)
pUC118 DNA (Cat. #3318)
pUC119 DNA (Cat. #3319)
Endonuclease cut pUC118 DNA (BAP treated) (Cat. #3320 - 3324)
X-Gal (5-Bromo-4-Chloro-3-Indolyl-$\beta$-D-Galactoside) (Cat. #9031)