TaKaRa

E. coli HST08 Premium Electro-Cells

Cat.# 9028

Manual

I. Contents

TaKaRa E. coli HST08 Premium Electro-Cells	50 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 HST08 Premium Electro-Cells are specially prepared by Takara to be highly amenable to electroporation. Electroporation is used to transfer DNA into cells by perforating the cytoplasmic membrane with a high voltage pulse.
In addition, *E. coli* HST08 Premium Electro-Cells lack the genes necessary for digesting foreign methylated DNA: *mrr*, *hsd*RMS, *mcr*BC, and *mcr*A. These properties make these competent cells useful in a wide range of applications such as methylated DNA cloning, genetic library construction, and BAC-based long-length genomic libraries. Even when working with very large plasmids, the transformation efficiency and colony growth rates remain very high*. Effective DNA cloning and genetic library construction with insert DNA larger than 10 kbp is possible if this product is used with TaKaRa's 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 adding X-Gal to the media, thus using the α -complementarity to β -galactosidase of the competent cell to visualize vector-insert containing transformants.

X-Gal：5-Bromo-4-Chloro-3-Indolyl-β -D-Galactoside

III. Protocol（Transformation with a Plasmid Vector）

（1） Thaw 50 μl of *E. coli* HST08 Premium Competent Electro-Cells on ice just before use.

（2） Add 1 - 2 μl of DNA solution＊ into the thawed cell suspension.

＊：When the sample DNA solution contains salt(s), dilute it with TE buffer or distilled sterilized water. Alternatively, desalination by ethanol precipitation is recommended. Ethanol precipitation should be performed when the sample DNA is prepared for use with the TaKaRa DNA Ligation Kit (Ver.1 and Ver. 2).

（3） Transfer the mixture of cells and DNA to a cold 0.1 cm electroporation cuvette.

（4） After applying a pulse＊, immediately add 1 ml of SOC medium (prechilled on ice).

＊：Takara uses BIBIO-RAD's Gene Pulser. The electrical conditions are 1.5 kV, 25 μ F, and 200 Ω , or 1.5 kV, 10 μ F, and 600 Ω under the voltage conditions in the provided on the lot card.

（5） Incubate at 37℃ for 1 hour with shaking at 160 ～ 225 rpm.

（6） Plate an appropriate amount of culture.*
（7） 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. For 50 μl of competent cells, use no more than 10 ng of high purity DNA, or the transformation efficiency may be reduced.
3. If you change the quantity of competent cells electroporated, it may be necessary to reevaluate the conditions.
4. L-broth or φ b-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.
   - ψ b-broth： 5 g Bacto yeast extract, 20 g Bacto tryptone, 5 g $MgSO_4$・$7H_2O$, 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 μl/100 ml agar media.
8. Once the 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
10 pg of pUC19 plasmid was transformed according to section III and transformants were selected on a L-plate containing ampicillin. The resulting transformation efficiency was 1×10^9 colonies / μg•pUC 19 plasmid.

［2］Confirmation of β -galactosidase α -complementation.
When transformed with a pUC19 plasmid, blue colonies appeared on a L-agar plate containing 100 μ gl/ml ampicillin, and 40 μ 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*, λ –

VII. Cell density

> 1×10^{10} bacteria/ml

VIII. Storage

- 80 ℃

Warning: Store at -80℃ or lower. If the storage temperature is not maintained consistently, the transformation efficiency will 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) Dower, W.J., Miller, J.F. and Ragsdale, C.W. (1988) *Nucl.Acids Res*., 16, 6127.
2) Bottger, E.C. (1988) *Biotechniques*, 6, 878.

X. Related Products

E. coli HST08 Premium Competent Cells (Cat.# 9128)
TaKaRa DNA Ligation Kit LONG（Cat.# 6024）
X-Gal（Cat.# 9031）
IPTG (Cat.# 9030)

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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