Cat. # 9028

For Research Use

TaKaRa

E. coli HST08 Premium Electro-Cells

Product Manual

v202012Da

Table of Contents

I. Description

E. coli HST08 Premium Electro-Cells are specially prepared to be highly appropriate 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*, *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 BAC-based long-length genome 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 kb is possible if this product is used with 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 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

II. Components

E. coli HST08 Premium Electro-Cells	50 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 will 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

(1) Thaw 50 μl of *E. coli* HST08 Premium Electro-Cells on ice just before use.
(2) Add 1 - 2 μl of DNA solution＊1 into the thawed cell suspension.
(3) Transfer the mixture of cells and DNA to a cold 0.1 cm electroporation cuvette.
(4) After applying a pulse＊2, immediately add 1 ml of SOC Medium (prechilled on ice).
(5) Incubate at 37℃ for 1 hour with shaking at 160 - 225 rpm.
(6) Plate an appropriate amount of culture＊3.
(7) Place plates in a 37℃ incubator and grow overnight.

＊1 When the sample DNA solution contains salt(s), dilute it with TE buffer or sterile purified 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.
＊2 Takara Bio uses BIO-RAD MicroPulser and the electrical condition is 1.5 kV. In the case of BIO-RAD Gene Pulser, standard electrical conditions are 200 Ω, 25 μF and 1.5 kV.
＊3 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 (4).

【 Please read before proceeding 】

1. Place a tube of Electro-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 Electro-Cells, use no more than 10 ng of high purity DNA, or the transformation efficiency may be reduced.
3. For 50 μl of Electro-Cells, use no more than 5 μl of DNA solution, or the transformation efficiency may be reduced.
4. If you change the quantity of competent cells electroporated, it may be necessary to reevaluate the conditions.
5. L-broth or φb-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 1 N NaOH and autoclave.

   φb-broth :

Ingredient	per liter water
Tryptone	20 g
Yeast extract	5 g
$MgSO_4 \cdot 7H_2O$	5 g

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

6. When using X-Gal, follow the procedures described as below:
   Add 20 mg/ml X-Gal (dissolved in dimethylformamide) at a ratio of 200 - 300 μl/100 ml agar media.
7. 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 IV and transformants were selected on a L-plate containing ampicillin. The resulting transformation efficiency was > 1 x 10^9 colonies / μg pUC19 plasmid.
2. Confirmation of β-galactosidase α-complementation.
   When transformed with a pUC19 plasmid, blue colonies appeared on a L-agar plate containing 100 μgl/ml ampicillin, and 60 μg/ml X-Gal.

VI. Genotype

E. coli HST08 Premium :
F^-, *endA1*, *supE44*, *thi-1*, *recA1*, *relA1, gyrA96*, *phoA*, Φ80d *lacZ*ΔM15, Δ (*lacZYA - argF*) *U169*, Δ (*mrr - hsdRMS - mcrBC*), Δ*mcrA*, λ^-

VII. Cell density

> 1 x 10^{10} bacteria/ml

VIII. References

1) Dower W J, Miller J F, and Ragsdale C W. *Nucl Acids Res*. (1988) **16**: 6127.
2) Bottger E C. *Biotechniques*. (1988) **6**: 878.

IX. Related Products

E. coli HST08 Premium Competent Cells (Cat. #9128)
TaKaRa DNA Ligation Kit LONG (Cat. #6024)
X-Gal (5-Bromo-4-Chloro-3-Indolyl-β-D-Galactoside) (Cat. #9031)
pUC19 DNA (Cat. #3219)