PRODUCT INFORMATION

日本語データシート

Product Name: JetGiga Competent Cell (DH5α)
Code No.: DS230
Size: 100 µl × 10
Competency: >1 × 10^9 CFU/µg (pUC19)
Supplied Product: SOC medium, 1 ml ×10

This product is for research use only

Description:

JetGiga Competent Cell (DH5α) has the following features: "Rapid transformation (6 minutes)," "extremely high efficiency (>1 × 10^9 CFU/µg)," and "high stability against a freeze–thaw cycle" (Figure 2)." It gives satisfactory results even when dispensed to any volume and refrozen, due to this freeze–thaw stability (see Dispensing Procedure).

JetGiga Competent Cell (DH5α) was prepared from *E. coli* DH5α strain (one of the standard strains for molecular biology applications) by using an advanced chemical procedure. The DH5α cells possess a mutation of φ80*lacZ*ΔM15 and lack *laqIq* gene, which allows blue-white color screening of transformants with X-gal (IPTG is not required).

Genotype of *E coli* Strain DH5α:

*supE*44, Δ*lac*U169(φ80*lacZ*ΔM15), *hsdR*17, *recA*1, *endA*1, *gyrA*96, *thi*-1, *relA*1

Quality Control:

A transformation was performed using 0.2 ng of supercoiled pUC19 plasmid, according to the method described in the Product Information. The transformants were plated on LB plates containing 50 µg/ml ampicillin. The efficiency was confirmed to be greater than 1 × 10^9 CFU/µg.

Storage Conditions:

The product is stable at −80ºC, with little or no loss of transformation efficiency for up to 12 months from the date of receipt.

The competent cells are sensitive to variation in temperature, and should therefore be stored at −80ºC. Upon receipt, store the competent cells in a freezer at −80ºC directly from the dry ice shipping box and store the SOC medium at room temperature or at −80ºC.

Note:

If antibiotics other than ampicillin (such as kanamycin and tetracycline) are used for selection, an inadequate number of colonies may be obtained due to the rapid procedure (no outgrowth - recovery process).

Therefore, when using antibiotics other than ampicillin, perform the *additional step in the transformation procedure. (see Transformation Procedure and Figure 1).

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Transformation:

●Materials to be supplied by the user:

- LB plates with antibiotic
- 42°C water bath
- 37°C incubator
- Ice bucket with ice
- Sterile spreaders

If blue-white screening is required to select transformants,

- 20 mg/ml X-Gal in dimethylformamide (DMF)

● Transformation procedure:

1) Thaw one tube of JetGiga Competent Cell (DH5α) on ice. One tube contains 100 µl of cells for each transformation.

2) Add the DNA sample* directly into the competent cells and mix by flicking** the tube about 10 times.

*The volume of the DNA sample should not exceed 5% of that of the competent cells (i.e., for 100 µl of the competent cells, use ≤5 µl).

**Do not vortex

3) Incubate the tube on ice for 5 minutes.

4) Heat-shock the cells by placing the tube in 42°C water bath for 30 seconds*. Do not mix or shake.

*The appropriate heat-shock time depends on the volume of the competent cells.

Volume/tube	Heat-shock time
50 – 100µl	30 seconds
<50 µl	20 seconds

5) Remove the tube from the 42°C water bath and place it on a tube rack for cooling.

Ampicillin selection→ Step 7)

Other than ampicillin selection→ Step 6) (Additional step)

Additional step:

6) Transfer the cells to a 15-ml sterilized culture tube containing 0.9 ml of SOC medium (pre-warmed from room temperature to 37°C). Culture the cells at 37°C for 60 min in a shaker.

7) Spread an aliquot of the cells onto an LB agar plate containing appropriate antibiotic.

If blue-white color screening is required, spread 25 µl of 20 mg/ml X-Gal onto an LB agar plate and allow the reagent to absorb for 30 minutes before inoculating the cells. As DH5α does not possess *lacI*^{q}, IPTG is not required for blue-white screening.

When diluting the transformation solution, use an appropriate medium (e.g., SOC, SOB, LB).

8) Incubate the plate at 37°C overnight.

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Dispense:

JetGiga Competent Cell (DH5α) has high stability against the freeze–thaw cycle. Its transformation efficiency remained over 1×10^9 CFU/μg when refrozen after thawing (see Figure 2).
For dispensing JetGiga Competent Cell (DH5α), perform the following procedure:

● Materials to be supplied by the user:

- Sterile 1.5-ml tubes
- Ice-water bucket
- Deep freezer (−80ºC)
- Sterile pipette tips
- Thermometer
- Freezer (−20ºC)

●Dispensing procedure:

A repeated freeze–thaw cycle may remarkably reduce the transformation efficiency of the cells.
Do not freeze–thaw the competent cells more than twice.

1) Prepare the ice-water (add water until ice is soaked) and wait until the ice-water is sufficiently chilled*
*Confirm that the water is at 0ºC using a thermometer.

2) Chill several tubes and pipette tips in a freezer at −20ºC.

3) Thaw JetGiga Competent Cell (DH5α) on ice-water. The time required for thawing 100 μl of the competent cells is about 4 minutes.

4) Dispense aliquots* of the competent cell suspension into the pre-chilled tubes using the chilled pipette tips within 5 minutes**
*Dispensing volume is recommended to be >20 μl/tube, as the competent cells of which volume is <20 μl/tube is sensitive to heat-shock at 42ºC.
**After thawing the competent cells on ice, the cells tend to lose their transformation efficiency gradually. Therefore, it is preferable to dispense the cells as soon as possible.

5) Freeze the cells in a deep freezer (−80ºC).

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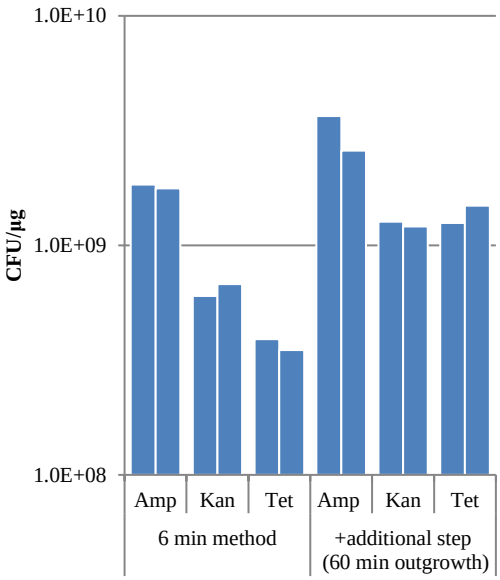

Figure 1: Transformation efficiency of JetGiga Competent Cell (DH5α) with various antibiotics.

JetGiga competent cell (DH5α) was transformed with pBR322 (Ampicillin^R, Tetracycline^R) or pACYC177 (Kanamycin^R). The transformation efficiency was then compared between the 6-min method and the 6-min method + 37ºC outgrowth for 60 min (+additional step).

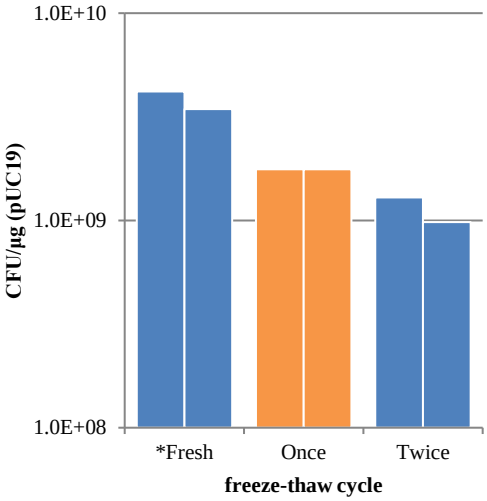

Figure 2: Effect of repeated freeze–thaw cycles.

Thawed JetGiga Competent Cell was refrozen in a deep freezer. Then, the refrozen competent cells were transformed with pUC19 plasmid by the JetGiga method.

*Fresh: Initial thawing after purchase.

Reference:

Sambrook, J. and Russell, D.W. (2001) Molecular Cloning: A LaboratoryManual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Related Products:

DS210	Competent Cell JM109	DS220	Competent Cell DH5α
DS225	Jet Competent Cell (DH5α)		
DS255	Zip Competent Cell BL21(DE3)		