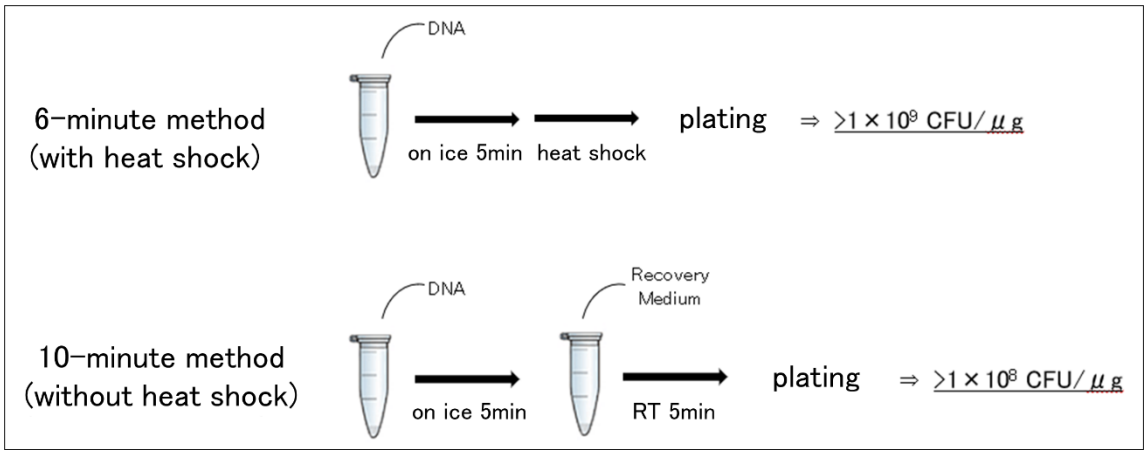
Product Name: DynaCompetent Cells JetGiga DH5α
Code No.: DS230
Size: 100 µl × 10
Supplied Product: Recovery Medium, 1 ml ×10

日本語データシート

This product is for research use only

Competency (pUC19):



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 transformation efficiency was confirmed to be greater than 1 × 10^9 CFU/µg in the 6-minute method (with heat shock), and greater than 1 × 10^8 CFU/µg in the 10-minute method (without heat shock).

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 Recovery Medium at 4ºC 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 on page 2).

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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:

Depending on the experimental content, you can choose between a 6-minute method with heat shock (>1 × 10^9 CFU/μg) or a 10-minute method without heat shock (>1 × 10^8 CFU/μg).

①6-minute method (with heat shock)

1) Thaw one tube of DynaCompetent Cells JetGiga 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 – 100 μl	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 Recovery Medium (pre-warmed from room temperature to 37°C). Culture the cells at 37°C for 60 minutes 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., Recovery Medium, SOC, SOB, LB).

8) Incubate the plate at 37°C overnight.

②10-minute method (without heat shock)

1) Thaw one tube of DynaCompetent Cells JetGiga 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 DNA sample should not exceed 5 % of that of competent cells (i.e., for 100 µl of competent cells, use ≦ 5 µl).

**Do not vortex

3) Incubate the tube on ice for 5 minutes.

Ampicillin selection→ Step 5)

Other than ampicillin selection→ Step 4) (Additional step)

Additional step:

4) Transfer the cells to a 15-ml sterilized culture tube containing 0.9 ml of Recovery Medium (pre-warmed from room temperature to 37°C). Culture the cells at 37°C for 60 minutes in a shaker.

5) Transfer the cells to a new 1.5 ml sterile tube containing 0.9 ml of Recovery Medium (room temperature or pre-warmed at 37°C), mix the tube contents by vortex for one second, and incubate the tube at room temperature for 5 minutes.

6) Spread all or an aliquot of the cells to an LB agar plate containing appropriate antibiotic.

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

Note: It is especially important to absorb these solutions prior to inoculating cells for kanamycin or tetracycline selection. Do not mix cells with solutions of these reagents before inoculating to a plate.

7) Incubate the plate at 37°C overnight.

Dispense:

DynaCompetent Cells JetGiga DH5α has high stability against the freeze–thaw cycle. Its transformation efficiency remained over 1×10^9 CFU/µg when refrozen after thawing.

For dispensing DynaCompetent Cells JetGiga 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) Chill several tubes and pipette tips in a freezer at −20ºC.
2) 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.
3) Thaw DynaCompetent Cells JetGiga 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).