Cat. # HB116

For Research Use

TaKaRa

Brevibacillus Competent Cells

Product Manual

v202104

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I. Description

Brevibacillus Competent Cells are host cells used for transformation with *Brevibacillus* expression vectors such as pNCMO2 DNA (Cat. #HB112), pNC-HisT DNA (Cat. #HB121), and others. *Brevibacillus* Competent Cells are prepared for transformation using the New Tris-PEG (NTP) method for chemical transformation of *B. choshinensis* strain SP3. With the NTP method, it is possible to obtain a transformation efficiency that is approximately equal to that obtained with electroporation. The NTP method also enables direct transformation of a DNA ligation solution without DNA purification steps including ethanol precipitation.

For instructions on the use of the *Brevibacillus* expression system, please refer to the manual for BIC System (Cat. #HB300) or *Brevibacillus* Expression System II (Cat. #HB200) for secretory protein expression and the manual for pNI DNA/pNI-His DNA (Cat. #HB131/HB132) for intracellular protein expression.

II. Components

Brevibacillus Competent Cells	100 µl x 10
MT Medium	1 ml x 10
Solution A	1 ml
Solution B	1 ml x 2

III. Materials Required but not Provided

1. Reagents

- Plasmid for target gene expression
- MTNm plates
- MT Liquid Medium
- Neomycin

Note: See Section V-1. for MTNm plate and MT Liquid Medium components and preparation.

2. Materials

- Sterile culture tubes
- Sterile microtubes
- Microcentrifuge
- Vortex
- Incubating orbital shaker

IV. Storage

-80℃

Note: Please store at -80℃ or less. Insufficient temperature control may lead to decreased transformation efficiency. Do not store in liquid nitrogen.

V. Protocol

1. Preparation

Prepare the following reagents and materials:

Plasmid for target gene expression
MTNm Plates＊1
MT Liquid Medium＊1
Sterile culture tubes＊2
Sterile microtubes

＊1 Medium Composition

MT Liquid Medium

Glucose＊3	10.0 g/L
Phytone Peptone	10.0 g/L
35%Ehrlich Bonito Extract	5.75 g/L
Yeast extract Blue label	2.0 g/L
$FeSO_4 \cdot 7H_2O$	10 mg/L
$MnSO_4 \cdot 4H_2O$	10 mg/L
$ZnSO_4 \cdot 7H_2O$	1 mg/L
$MgCl_2 \cdot 6H_2O$	4.1 g/L

Adjust to pH 7.0 with NaOH

＊3 Sterilize glucose and glucose-free media separately. Mix after sterilization.

MTNm Plates

Suspend 7.5 g of agar in 500 ml of MT Liquid Medium and sterilize using an autoclave. Let stand at room temperature until it has cooled to approximately 50℃ and then add neomycin solution (50 mg/ml stock solution) to a final concentration of 50 μg/ml. Mix gently and dispense into plates.

＊2 e.g., 14-ml round-bottom sterile tube (falcon tube).

2. NTP Transformation Method

(1) Thaw Solution A, Solution B, and MT Medium.
(2) Transfer only the number of tubes of *Brevibacillus* Competent Cells needed for transformation from storage, and keep on dry ice/ethanol.
(3) Thaw the *Brevibacillus* Competent Cells quickly (approximately 30 seconds) in a 37℃ water bath.
(4) Centrifuge the cells (12,000 rpm, 30 seconds to 1 minute) to form a cell pellet and remove the supernatant with a micropipette.

Perform the following procedures at room temperature.

(5) Mix the plasmid DNA solution (in a volume of 5 μl or less)＊1 with 50 μl of Solution A.
(6) Add all of the DNA solution to the Brevibacillus cell pellet (from step 4) and vortex to completely suspend the pellet.
(7) Allow to stand for 5 minutes at room temperature.
(8) Add 150 μl of Solution B (PEG solution)＊2 and vortex until the solution is uniform (5 - 10 seconds).
(9) Centrifuge the cells (5,000 rpm, 5 minutes) and remove the supernatant.
(10) Centrifuge briefly (5,000 rpm, 30 seconds) and remove the supernatant completely.
(11) Add 1 ml of MT Medium and suspend completely with a micropipette.
(12) Transfer the medium containing the cells into a culture tube, then incubate for 2 hours at 37℃ in an orbital shaker (120 rpm).
(13) Use a sterile inoculating loop to remove a small sample from the culture. Streak on the MTNm plates and culture overnight at 37℃.
(14) Select isolated colonies for plasmid analysis or protein expression.

＊1 When DNA ligation solution is used, mix 5 μl of the reaction solution with Solution A. When using purified plasmids, use 10 - 100 ng.
＊2 Solution B (PEG solution) is highly viscous - use a 1,000 μl micropipette and pipette slowly.

VI. Transformation Efficiency

Transformation with 10 ng of pNY326 plasmid was performed according to the protocol and colonies that formed on a MTNm plate were selected. Transformation efficiency was $>10^5$ transformants/μg pNY326 plasmid.

VII. Genotype

An essential gene for spore formation is disrupted in *B. choshinensis* SP3; therefore sterilization of transformants may be performed using standard autoclave conditions. Furthermore, trace activity of an intracellular protease (*imp*) and extracellular protease (*emp*) have been disrupted to prevent degradation of expressed proteins.

VIII. Related Products

BIC System (Cat. #HB300)
Brevibacillus Expression System II (Cat. #HB200)
pNCMO2 DNA (Cat. #HB112)
pNC-HisT DNA (Cat. #HB121)
pNC-HisF DNA (Cat. #HB122)
pNC-HisE DNA (Cat. #HB123)
pNI DNA (Cat. #HB131)
pNI-His DNA (Cat. #HB132)

IX. Notice: Living Modified Organism

Brevibacillus Competent Cells (Cat. #HB116) , BIC system (Cat. #HB300), and *Brevibacillus* Expression System II (Cat. #HB200) include a genetically "Living Modified Organism (LMO)" defined in "The Cartagena Protocol on Biosafety". The supplied *Brevibacillus* Competent Cells in these kits contain partial sequences of the 2 μm plasmid derived from *Saccharomyces cerevisiae*.
Please follow the guidelines, laws, and regulations specific to your country and ensure safe handling, storage, transport, and disposal.

This product is developed and manufactured by Higeta Shoyu Co., Ltd. and sold by Takara Bio Inc.