ig™ *Lactococcus lactis* Cells
Electrocompetent Cells

Description

Intact Genomics Electroporation Competent Lactococcus lactis Cells are customer strains optimized for the highest transformation efficiencies which is ideal for applications requiring high transformation efficiencies, such as with cDNA or gDNA library construction.

Specifications

Competent cell type:	Electrocompetent
Species:	*L. lactis*
Strain:	IL 1403 or MG1363
Format:	Tubes
Transformation efficiency:	≥ 1 x 10^6 cfu/µg pNZ8148 DNA
Blue/white screening:	No
Shipping condition:	Dry ice

Reagents Needed for One Reaction

ElectroCompetent Lactococcus lactis Cells :	25 µl
DNA (or pNZ8148 Control, 50 ng/µl):	1 µl
Recovery medium:	1 ml

Storage

ElectroCompetent Lactococcus lactis Cells :	-80 ºC
pNZ8148 control DNA:	-20 ºC
Recovery medium:	4 ºC

Quality Control

Transformation efficiency is tested by using the pNZ8148 control DNA supplied with the kit and using the protocol in this manual. Transformation efficiency should be ≥1 x 10^6 CFU/µg pNZ8148 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines

Follow these guidelines when using Electroporation Competent Lactococcus lactis Cells:

- Handle competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting.
- Thaw competent cells on ice, and transform cells immediately following thawing. After adding DNA, mix by tapping the tube gently. Do not mix cells by pipetting or vortexing.

Note: A high-voltage electroporation apparatus such as Bio-Rad Gene Pulser II #165-2105, capable of generating field strengths of 16 kV/cm is required.

Calculation of Transformation Efficiency

Transformation Efficiency (TE) is defined as the number of colony forming units (cfu) produced by transforming 1µg of plasmid into a given volume of competent cells.

TE = Colonies/µg/Dilution

Transform 1 µl of (50 ng/µl) pNZ8148 control plasmid into 25 µl of cells, add 976 µl of Recovery Medium. Dilute 100 µl of this in 900 µl of Recovery Medium and plate 50 µl. Count the colonies on the plate in two days. If you count 300 colonies, the TE is calculated as follows:

Colonies = 300
µg of DNA = 0.05
Dilution = 50/1000 x 100/1000 = 0.005
TE = 300/.05/.005 = 1.2x10^6

Transformation Protocol

Use this procedure to transform Electroporation Competent Lactococcus lactis Cells. Do not use these cells for chemically transformation.

1) Place sterile cuvettes and microcentrifuge tubes on ice.
2) Remove competent cells from the -80 °C freezer and thaw completely on wet ice (10-15 minutes).
3) Aliquot 1 µl (1 ng -10 µg) of DNA to the chilled microcentrifuge tubes on ice.
4) When the cells are thawed, add 25 µl of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pNZ8148 control, add 1 µl of (50 ng/µl) DNA to the 25 µl of cells on ice. Mix well by tapping. Do not pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.
5) Pipette 26 µl of the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Quickly flick the cuvette downward with your wrist to deposit the cells across the bottom of the well and then electroporate.
6) Immediately add 976 µl of Recovery Medium or any other medium of choice to the cuvette, pipette up and down three times to re-suspend the cells. Transfer the cells and Recovery Medium to an Eppendorf tube.
7) Seal the closed tube caps with parafilm and quickly warm tubes to 37 °C using a water bath.
8) Incubate tubes at 37 °C for 3 hours with no shaking.
9) Dilute the cells as appropriate then spread 20-200 µl cells onto a pre-warmed selective plate. For the pNZ8148 control, dilute the cells 1/10 and plate 50 µl of diluted transformants onto an MRS plate containing 10 µg/ml chloramphenicol. Use sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
10) Incubate the plates for 2 days at 37 °C under anaerobic conditions.

Electroportation settings

Mode	Exponential protocol
Voltage (V)	2,500 V
Capacitance	25 uFD
Resistance	400 ohms
Cuvette	2 mm