BSL2 facility is required for purchase and use of this product

Staphylococcus aureus RN4220 ElectroCompetent Cells

Intact Genomics®

Catalog #	1294-40
Package Size	5x200 µl

Description

Intact Genomics (ig®) *Staphylococcus aureus* (*S. aureus*) RN4220 electrocompetent cells are optimized to provide high transformation efficiencies making them ideal for various applications. This strain is commonly used in studies involving virulence, resistance, metabolics and more. *Staphylococcus aureus* RN4220 is characterized by a mutation in the sau1 hsdR genes, making it restriction deficient and hence an excellent intermediate cloning host.[1]

Specifications

Competent cell type:	Electrocompetent
Species:	*S. aureus*
Strain:	RN4220
Format:	Tubes
Transformation efficiency:	≥1.0 x 10^5 cfu/µg
Blue/white screening:	No
Shipping condition:	Dry ice

Reagents Included

- ig® *Staphylococcus aureus* (*S. aureus*) RN4220 Electrocompetent Cells
- Control plasmid DNA
- Recovery medium

Storage

• ig® *S. aureus* ElectroCompetent cells:	-80 ºC
• Control plasmid DNA (50ng/µl):	-20 ºC
• Recovery medium:	4 ºC

Quality Control

Transformation efficiency is tested by using the control plasmid DNA supplied with the kit and using the protocol in this manual. Transformation efficiency should be ≥1.0 x 10^5 cfu/µg . Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines

Follow these guidelines when using ig® *S. aureus* ElectroCompetent 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.

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/Plated
Transform 1 µl of (500 ng/µl) of the control plasmid into 25 µl of cells, add 500 µl of Recovery Medium. Recover for one hours and plate 100 µl. Count the colonies on the plate in two days. If you count 1000 colonies, the TE is calculated as follows:

Colonies = 1,000
µg of DNA = 0.05
Dilution = 100/500 = 0.2
TE = 1000/.05/.2= $1x10^5$

Transformation Protocol

Use this procedure to transform ig® *Staphylococcus aureus* ElectroCompetent 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 (10pg -1 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 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 500µl 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) Incubate tubes at 37 °C for 1 hours at 200 RPM.
8) Dilute the cells as appropriate then spread 20-200 µl cells onto a pre-warmed selective plate. For the control plasmid , you may plate 100 µl of undiluted transformation mix onto a YT plate containing chloramphenicol 12.5µg/µl). Use sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
9) Incubate the plates for overnight at 37 °C.

Electroportation settings

Mode	Exponential protocol
Voltage (V)	1,800 V
Capacitance	25 uFD
Resistance	200 Ohms
Cuvette	1 mm

Related Products

- T4 DNA Ligase (Cat.# 3212)
- igFusion Cloning Kit (Cat.# 4111)
- i7® High Fidelity DNA Polymerase (Cat.# 3254)
- E. coli and other competent cells

Technical Support

Intact Genomics is committed to supporting the worldwide scientific research community by supplying the highest quality reagents. Each new lot of our products is tested to ensure they meet the quality standards and specifications designated for the product.

Please follow the instructions carefully and contact us if additional assistance is needed. We appreciate your business and your feedback regarding the performance of our products in your applications.

References

1. Nair, D., Memmi, G., Hernandez, D., Bard, J., Beaume, M., Gill, S., Francois, P., & Cheung, A. L. (2011). Whole-Genome Sequencing of Staphylococcus aureus Strain RN4220, a Key Laboratory Strain Used in Virulence Research, Identifies Mutations That Affect Not Only Virulence Factors but Also the Fitness of the Strain. Journal of Bacteriology, 193(9), 2332–2335. https://doi.org/10.1128/jb.00027-11