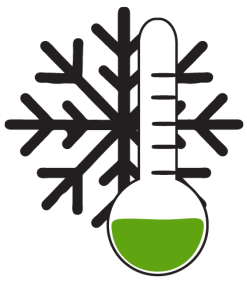
ER2738 Phage Display Chemically Competent Cells

Manual



Important!

-80°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE

Catalog #1017-12 and 1017-24

Intact Genomics, Inc.

www.intactgenomics. com

V2023 Rev 0

Description:

Intact Genomics (ig®) ER2738 Phage Display Chemically Competent Cells are suitable for protein expression and preparation of antibody or peptide phage display libraries. ER2738 cells are also useful for protein expression, M13 phage work, general cloning, and blue/ white screening.

Reagents Included:

- ig® ER2738 phage display chemically competent cells
- pUC19 Control
- Recovery medium

Product Storage:

ig® ER2738 phage display chemically competent cells: -80 ºC

pUC19 control DNA: -20 ºC

Recovery medium: 4 ºC

Genomic Features:

Intact Genomics ER2738 phage display chemically competent cells have the following features:

- >1 x 10^{10} cfu/µg efficiency with electroporation.
- Amber suppressor strain (glnV)

Genotype:

[F'proA+B+ lacIq Δ(lacZ)M15 zzf::Tn10 (tetr)] fhuA2 glnVΔ(lac-proAB) thi-1Δ(hsdS-mcrB)5

Quality Control:

Transformation efficiency is tested by using the pUC19 control DNA supplied with the kit and using the protocol given below. Transformation efficiency should be >1 x 1010 CFU/µg pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

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 (10 pg/µl) pUC19 control plasmid into 50 µl of cells, add 950 µl of Recovery Medium. Dilute 10 µl of this in 990 µl of Recovery Medium and plate 50 µl. Count the colonies on the plate the next day. If you count 50 colonies, the TE is calculated as follows:
Colonies = 50
µg of DNA = 0.00001
Dilution = 50/1000 x 10/1000 = 0.0005
TE = 50/.00001/.0005 = $1.0x10^{10}$

Transformation Protocol:

Use this procedure to transform ER2738 phage display chemically competent cells . We recommend verifying the transformation efficiency of the cells using the pUC19 control DNA supplied with the kit. Do not use these cells for electroporation.

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 pg-10 ng) 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 pUC19 control, add 1 µl of (10 pg/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 974 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 a culture tube.
7) Incubate tubes at 37 °C for 1 hour at 210 rpm.
8) Dilute the cells as appropriate then spread 20-200 µl cells onto a pre-warmed selective plate. For the pUC19 control, plate 50 µl of diluted transformants onto an LB plate containing 100 µg/ml ampicillin. Use sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
9) Incubate the plates overnight at 37 °C.

5 Minute Transformation Protocol:

The following procedure results in only ~10% of the transformation efficiency as the protocol listed above.

1) Remove competent cells from the -80 °C freezer and thaw in your hand.
2) Aliquot 1-5 µl (1 pg-100 ng) of DNA to the microcentrifuge tubes. ***Do not*** pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.
3) Incubate the cells with DNA on ice for 2 minutes.
4) After 2 minute ice incubation, heat shock the cells at 42 °C for 45 seconds.
5) Transfer the tubes to ice for 2 minutes.
6) Add 950 µl of Recovery Medium at room temperature or any other medium of choice to each tube. Immediately spread 50 µl to 200 µl from each transformation on pre-warmed selection plates. We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, plate 50 µl on an LB plate containing 100 µg/ml ampicillin. Use sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
7) Incubate the plates overnight at 37 °C.

General Guidelines:

Follow these guidelines when using Intact Genomics ER2738 phage display chemically competent 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.

Related Products

- TG1 Phage Display Electrocompetent Cells (Cat#1264-24)
- SS320 Phage Display Electrocompetentt Cells (Cat#1264-24)
- Taq DNA Polymerase 2x Premix(Cat.# 3249)
- T4 DNA Ligase(Cat.# 3212)
- ig® 10B Chemically Competent Cells(Cat.# 1011-12)

Technical Support

Intact Genomics (IG®) is dedicated to customer satisfaction regarding the use of our products for your research needs. We test our products thoroughly to ensure they conform to the highest quality standards and provide excellent results when following the protocol's specifications. Please follow the protocol information provided in this manual carefully and contact our customer and technical support team with any questions or comments you may have regarding this or our other products.

IG Technical Support

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