Ingenio® Electroporation Solution for *CRISPR/Cas9 Ribonucleoprotein (RNP) Delivery*

Instructions for use with MIR 50108, 50109, 50110, 50111, 50112, 50113, 50114, 50115, 50116, 50117, 50118, 50119

SPECIFICATIONS

Storage	Store Ingenio® Electroporation Solution at 4°C.
Product Guarantee	1 year from the date of purchase, when properly stored and handled.

CRISPR/CAS9 RIBONUCLEOPROTEIN (RNP) ELECTROPORATION PROTOCOL

Transient RNP Electroporation Protocol

A. Plate cells

1. Approximately 18-24 hours before electroporation, passage cells to attain an optimal cell density at the time of electroporation (i.e. ~80% confluent for most cell types).

   For adherent cells: Seed cells at a density of 0.8 - 3.0 x 10^5 cells/ml
   For suspension cells: Seed cells at a density of 0.5 - 1.0 x 10^6 cells/ml.
2. Incubate cell cultures overnight.

B. Prepare Ingenio® Solution/RNP/cell mixture (Immediately before electroporation)

1. Warm Ingenio® Electroporation Solution, trypsin-EDTA (if needed) and complete growth medium to room temperature.
2. Harvest cells as required per cell type. Count cells to determine cell density/ml.
3. Determine the total electroporation volume required to perform the desired number of electroporations.
   For 0.2 cm cuvettes: Multiply the required number of electroporations by 0.1 ml
   For 0.4 cm cuvettes: Multiply the require number of electroporations by 0.25 ml
4. Using the harvested cell density determined in step B2, calculate the cell volume required for all electroporations according to the formula:

   $$\text{Cell volume (ml)} = \frac{\text{Final cell density/ml}}{\text{Harvested cell density/ml}} \times \text{Total electroporation volume (ml)}$$

   For adherent cells: Use a final cell density of 1 - 5 x 10^6 cells/ml
   For suspension cells: Use a final cell density of 10 x 10^6 cells/ml.
5. Pipette the cell volume (determined in step B4) of harvested cells into a new tube and centrifuge at 300 *x g* for 5 minutes. Aspirate the supernatant.
6. During centrifugation, add pre-warmed complete culture medium to a new culture dish to accept cells following electroporation.
7. Prepare the Ingenio® Solution/cell mixture by resuspending cells from step B5 in the electroporation volume (calculated in step B3) of Ingenio® Electroporation Solution.
8. To a microcentrifuge tube, add the volume of gRNA required to attain a 1500 nM final concentration in cuvette. NOTE: If using a two-part gRNA, combine the trans-activating RNA (tracrRNA) and target specific CRISPR RNA (crRNA) and incubate 5 minutes at room temperature to anneal.

   Example (0.2 cm cuvette): Combine 3 µl each of tracrRNA and crRNA (50 µM stock solutions) per 100 µl volume.
   Example (0.4 cm cuvette): Combine 7.5 µl each of tracrRNA and crRNA (50 µM stock solutions) per 250 µl volume.

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9. Add the Cas9 protein (750 nM final concentration in cuvette) to the gRNA mixture prepared in step B8.
   Example (0.2 cm cuvette): Add 2.5 µl of the Cas9 protein (30 µM stock solution).
   Example (0.4 cm cuvette): Add 6.25 µl of the Cas9 protein (30 µM stock solution).
10. Incubate the gRNA/Cas9 protein mixture at room temperature for 5 minutes.
11. Prepare the Ingenio®/RNP/cell mixture by adding the RNP complex (step B9) to the Ingenio®/cell mixture prepared in Step B7. NOTE: The 2:1 ratio of gRNA:Cas9 protein (1500 nM gRNA:750 nM Cas9; final concentration in cuvette) in this protocol is a starting point for RNP electroporation. Further ratio optimization may be required for some cell types.
12. Mix gently but thoroughly. Do not create air bubbles in the mixture.

C. Perform Electroporation

1. Aliquot the Ingenio®/RNP/cell mixture into cuvettes for electroporation.
   For 0.2 cm cuvettes: Pipet 100 µl Ingenio® Solution/RNP/cell mixture per cuvette
   For 0.4 cm cuvettes: Pipet 250 µl Ingenio® Solution/RNP/cell mixture per cuvette
2. Electroporate the cells at room temperature. NOTE: The optimal pulse conditions or program settings will vary depending on the cell type and electroporator used. Refer to the Ingenio® Electroporation Solution Full Protocol for recommended pulse conditions based on cell type. If your cell type is not listed, the correct settings should be determined experimentally.
3. Transfer the electroporated cells into the culture dish (prepared in step B6).
   Example: Transfer 100 µl of electroporated cells per well of a 12-well plate.
   NOTE: Users should determine their own post-electroporation best cell culture density depending on the cell type, nucleic acid and post-electroporation incubation period.
4. Incubate the electroporated cells in appropriate culture medium for 48-72 hours or as required. A culture medium change may be necessary for longer incubations.
5. Harvest cells and assay as required.

Reagent Agent®

Reagent Agent® is an online tool designed to help determine the best solution for nucleic acid delivery based on in-house data, customer feedback and citations.

Learn more at: mirusbio.com/ra

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