



## CRISPR Editing of Immortalized Cell Lines with RNPs using Neon Electroporation [↗](#)

Synthego<sup>1</sup>

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Working  
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### ABSTRACT

This protocol describes how to deliver ribonucleoprotein (RNP) complexes that consist of purified Cas9 nuclease duplexed with chemically modified synthetic single guide RNA (sgRNA) to immortalized adherent or suspension cells. RNP delivery is accomplished using the Thermo Fisher Neon<sup>TM</sup> Transfection System. A reference for electroporation settings for a wide variety of cell types is included. A option for knock-in is included. Chemically modified sgRNAs are designed to resist exonucleases and innate intracellular immune cascades that can lead to cell death. Synthego chemically modified synthetic sgRNAs are of exceptional purity and consistently drive high editing frequencies.

### TAGS

CRISPR/Cas9

sgRNA

Show tags

### EXTERNAL LINK

<https://www.synthego.com/resources/immortalized-cell-neon-electroporation-protocol>



Immortalized Cell Neon  
Electroporation.pdf

### PROTOCOL STATUS

#### Working

Synthego uses this protocol and it is working.

### GUIDELINES

#### Abbreviations:

CRISPR: clustered regularly interspaced short palindromic repeats Cas9: CRISPR associated protein 9

sgRNA: single guide RNA

RNP: ribonucleoprotein

PCR: polymerase chain reaction

ICE: inference of CRISPR edits

FACS: fluorescence-activated cell sorting PBS: phosphate-buffered saline

TE: Tris EDTA

GFP: green fluorescent protein

#### Important Considerations

##### Working with RNA and RNPs

Wearing gloves and using nuclease-free tubes and reagents is recommended in order to avoid RNase contamination.

Always maintain sterile technique, and use sterile, filter pipette tips.

All Synthego reagents should be stored according to the manufacturer's recommendations.

Synthetic sgRNA should be dissolved in TE buffer and diluted to a working concentration using nuclease-free water. Please consult the [Synthego Quick Start Guide](#) for best practices related to dissolving and storing synthetic sgRNAs.

RNP complexes are stable at room temperature for up to 1 hour (may be stored at 4°C for up to one week, or at -20°C for up to 1 month). Note that RNPs stored at 4°C may become susceptible to contamination from microbial growth after long periods of time.

#### Optimized Protocols

For specific electroporation settings for your cell type, we suggest consulting the [Thermo Fisher Neon™ Transfection System Protocols and Cell Line Data](#). Optimization of editing efficiency for a specific cell type will require empirically determining the number of cells required, amount of Cas9 and ratio of sgRNA:Cas9. This guide is meant to provide a starting point for your CRISPR editing experiments.

#### Suggested Controls

| Control      | Description   | Purpose  |
|--------------|---|--|
| Mock         | Cells transfected without Cas9 and sgRNA                                  | Wild type sequence for comparison with experimental and other negative controls.<br>Control toxicity from RNP, cell death from electroporation or possible viability issues associated with editing the specific gene of interest. |
| Negative     | Cas9 complexed with a non-targeting sgRNA or no sgRNA                     | Ensure that there are no false positives due to contamination (no effect expected=wild type).  |
| Positive     | sgRNA with high editing efficiency (e.g., <i>CDC42BPB</i> , <i>RELA</i> ) | Ensure all reagents, protocol, and equipment are functioning (effect expected).  |
| Transfection | pMAX GFP (Lonza), GFP mRNA (SBI)  | Assess transfection efficiency (without the use of RNPs).  |

#### Timeline

| Pre-Electroporation |       | Setup & Electroporation   | Post-Electroporation |       |          |
|---------------------|-------|---------------------------|----------------------|-------|----------|
| Day 1               | Day 2 | Day 3                     | Day 4                | Day 5 | Day 6    |
| Seed Cells          |       | Prepare Destination Plate |                      |       | Analysis |
| Incubate (2 days)   |       | Assemble RNP Complexes    |                      |       |          |
|                     |       | Prepare Cells             |                      |       |          |
|                     |       | Transfect cells           |                      |       |          |
|                     |       | Incubate (3 days)         |                      |       |          |

#### Additional Information















For an up-to-date list of all Synthego Protocols and other resources, please visit [synthego.com/resources](https://synthego.com/resources)

For technical assistance, contact our Scientific Support Team:

Ph: 888.611.6883

Email: [support@synthego.com](mailto:support@synthego.com)

## MATERIALS

| NAME   | CATALOG #                 | VENDOR                   |
|--|---------------------------|--------------------------|
|  Chemically modified sgRNA  | Chemically modified sgRNA | Synthego                 |
|  Cas9 2NLS nuclease (S. pyogenes)   | Cas9 2NLS nuclease        | Synthego                 |
|  Positive control (optional); Recommended: human RELA sgRNA, CDC42BPB sgRNA     |                           | Synthego                 |
|  Transfection control (optional); Recommended: pMAX GFP (Lonza), GFP mRNA (SBI) |                           | Contributed by users     |
|  TE buffer (Included with Synthego sgRNA)                                       |                           | Synthego                 |
|  Nuclease-free water  | R0581                     | Thermo Fisher Scientific |
|  Cell counter  |                           | Thermo Fisher Scientific |
|  Normal growth medium (Cell-type dependent)                                   |                           | Contributed by users     |
|  TrypLE Express or preferred cell dissociation reagent                        |                           | Thermo Fisher Scientific |
|  1X PBS, cell culture grade   |                           | Thermo Fisher Scientific |
|  Microcentrifuge tubes  |                           | Eppendorf                |
|  Neon™ Transfection System  | MPK5000                   | Thermo Fisher Scientific |
|  Neon™ Transfection System 10 µL Kit  | MPK1025                   | Thermo Fisher Scientific |
|  Tissue culture plates  |                           | Thermo Fisher Scientific |

## SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for safety warnings and hazard information.

## Pre-Electroporation

- Subculture cells 2 days before electroporation and seed cells in an appropriately sized vessel so that they are 70-80% confluent on the day of transfection. Each electroporation reaction will require approximately  $1 \times 10^5$  -  $2 \times 10^5$  cells, depending on the cell type.

### NOTE

For cell type specific information, refer to [Thermo Fisher Neon™ Transfection System Protocols and Cell Line Data](#).

🕒 48:00:00 Subculturing cells

### Setup & Electroporation - Prepare Destination Plate

- 2 Pre-warm 1 ml of normal growth medium in each well of a 12-well cell culture plate per reaction.

📄 1 ml normal growth medium

#### NOTE

*This will serve as the destination plate after electroporation.*

### Setup & Electroporation - Assemble RNP Complexes

- 3 Prepare sgRNA stock at 30  $\mu\text{M}$  and Cas9 nuclease stock at 20  $\mu\text{M}$ , and store at  $-80^{\circ}\text{C}$  until use. 🧊  $-80^{\circ}\text{C}$

- 4 Synthego recommends sgRNA:Cas9 ratios between 3:1 and 9:1 for RNP formation. Below is an example using an sgRNA to Cas9 ratio of 9:1 for a single reaction (scale up appropriately). In appropriate plates/ tubes, assemble RNP complexes in the order shown below.

#### NOTE

*The sgRNA:Cas9 ratio may need to be determined empirically to achieve optimal editing efficiency.*

RNP Components, Molarity, & Volume

| Component           | Molarity                                | Volume                      |
|---------------------|---|-----------------------------|
| sgRNA               | 30 $\mu\text{M}$ (pmol/ $\mu\text{l}$ ) | 3 $\mu\text{l}$ (90 pmol)   |
| Cas9                | 20 $\mu\text{M}$ (pmol/ $\mu\text{l}$ ) | 0.5 $\mu\text{l}$ (10 pmol) |
| Resuspension buffer | -                                       | 3.5 $\mu\text{l}$           |
| Total volume        | -                                       | 7 $\mu\text{l}$             |

#### NOTE

**Knock-in Option:** to knock in small inserts (<50 bp), an ssDNA HDR Template can be added. The recommended length of each homology arm is at least 50 bp. Add 1  $\mu\text{l}$  60  $\mu\text{M}$  ssDNA HDR Template per reaction to each well. Optimization may be required. To knock in larger inserts and for more information on designing knock-in experiments, see [Tips and Tricks: Design and Optimization of CRISPR Knock-in Experiments](#).

- 5 Incubate RNPs for 10 minutes at room temperature.

🕒 00:10:00 Incubation

### Setup & Electroporation - Prepare Cells

- 6

#### NOTE

*For suspension cells: spin down cells before each aspiration of culture medium and washes. Skip*

steps 7 and 8 below.

Aspirate cell culture medium and wash cells 1-2 times with 1X PBS.

- 7 Add TrypLE Express and incubate the cells for ~5 minutes, or until they detach from the plate completely.

 **00:05:00 Incubation**

**NOTE**

*Do not shake or hit the flask to dislodge cells, as this may lead to clumping and inaccuracies in cell counting.*

- 8 Neutralize the dissociation reaction with 2X volume of normal growth medium.

- 9 Count cells to determine the cell density.

- 10 Transfer  $1-2 \times 10^6$  cells to a sterile microfuge tube. One tube will contain enough cells for ~10 transfections.

- 11 Centrifuge cells for 5 minutes at 500 x g. Aspirate medium.

 **00:05:00 Centrifugation**

- 12 Wash the cells once with 1X PBS.

- 13 Centrifuge cells for 5 minutes at 500 x g. Aspirate PBS.

 **00:05:00 Centrifugation**

- 14 Resuspend the cell pellet in 50 µl of resuspension buffer R (provided with Neon<sup>TM</sup> Transfection System 10 µl Kit).

 **50 µl resuspension buffer R**

**NOTE**

*Avoid storing the cell suspension for more than 15 minutes at room temperature, as this reduces cell viability and*

*transfection efficiency.*

- 15 Add 5 µl of cell suspension to each RNP solution (7 µl) to make 12 µl of cell-RNP solution per reaction.

 5 µl cell suspension

 7 µl RNP solution

#### Setup & Electroporation - Transfect Cells

- 16 Aspirate 10 µl of cell-RNP solution to a 10 µl Neon tip.

 10 µl cell-RNP solution

- 17 Electroporate using cell type optimized conditions.

#### NOTE

Refer to [Thermo Fisher Neon™ Transfection System Protocols and Cell Line Data](#).

- 18 Immediately transfer cells to a pre-warmed 12-well plate (prepared in step 2).

- 19 Incubate the cells for 2-3 days in a humidified 37°C/5% CO<sub>2</sub> incubator.

 36 °C Incubation

 48:00:00 Incubation

#### Post-Electroporation - Analysis

- 20 Extract DNA from cells.

- 21 Conduct analyses to determine editing efficiency: PCR, Sanger sequencing, and [ICE analysis](#). Next-Gen Sequencing, FACS, or functional tests may be conducted as alternatives.

#### NOTE

**Option:** If storing cells for future use is desired, split cells into two groups (one for analysis and one for cell culture).



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