

Electroporation of Dharmacon™ Edit-R™ Cas9 Nuclease protein NLS and synthetic guide RNA for genome engineering

Successful electroporation of Cas9 Nuclease protein with Nuclear Localization Signal (NLS), and synthetic guide RNA (single guide RNA (sgRNA) or crRNA complexed with tracrRNA) with subsequent gene knockout, requires careful optimization of delivery conditions with appropriate electroporation reagents and parameters for each cell line of interest. The protocol below assumes that experimental conditions have been optimized previously.

Materials required:

Edit-R CRISPR-Cas9 materials for gene editing can be ordered at gelifesciences.com/dharmacon

- Edit-R Cas9 Nuclease protein NLS, (Cat #CAS11728 (20 μM, 250 pmol), CAS11200 (20μM, 500 pmol), CAS11201 (20 μM, 1000 pmol) CAS11729 (40 μM, 500 pmol) or CAS11730 (40 μM, 1000 pmol))
- Synthetic targeting guide RNA (choose one):
 1. Edit-R synthetic crRNA and tracrRNA oligos:
 - a. crRNA, [predesigned for your gene of interest](#) in a variety of sizes, or designed and ordered using the [Dharmacon CRISPR RNA Configurator](#)
 - b. tracrRNA, 5, 20 or 50 nmol (Cat #U-002005-XX)

or
 2. Edit-R synthetic sgRNA, custom ordering using the [Dharmacon CRISPR RNA Configurator](#)



We recommend testing three to five guide RNA designs per gene of interest to identify the guide RNA with highest editing efficiency that also results in complete knockout of functional protein.

- Non-targeting control guide RNA (choose one):
 1. Edit-R crRNA Non-targeting Control (Cat #U-007501-XX, U-007502-XX, U-007503-XX, U-007504-XX, U-007505-XX)

or

 2. Custom synthetic sgRNA non-targeting control ordered using the [Dharmacon CRISPR RNA Configurator](#)

Electroporation experiments require standard cell culture reagents and instruments appropriate for maintenance of cells. The following additional materials are required but not supplied:

- Electroporation instrument
- Electroporation reagents (buffer, cuvettes, transfer pipettes)
- Multi-well tissue culture plates or tissue culture dishes
- Antibiotic-free complete medium: Cell culture medium (including serum and/or supplements) without antibiotic; recommended for maintenance and passaging of the cells of interest
- Assay(s) for detecting gene editing events in a cell population
- 10 mM Tris pH 7.4 nuclease-free buffer solution for resuspension of synthetic crRNA and tracrRNA (Dharmacon, Cat #B-006000-100)

General protocol for electroporation of Edit-R Cas9 Nuclease protein NLS and synthetic RNAs:

The following example provides a general protocol using electroporation to deliver Cas9 protein and guide RNA into cultured mammalian cells. Exact reagents, amounts and parameters for electroporation should be empirically determined through careful optimization in cells of interest, in accordance with electroporation instrument manufacturer's recommendations. The protocol below describes delivery conditions in U2OS cells (1×10^6) using the Lonza Nucleofector™ 2b instrument and is given for illustrative purposes only. All steps of the protocol should be performed in a laminar flow cell culture hood using sterile technique.

Cell plating

Optimal cell number for plating will vary with growth characteristics of specific cells and should be determined empirically.

1. Trypsinize and count cells.
2. Plate cells to achieve 70-80% confluence the next day. Cell densities greater than 80% may reduce electroporation efficiency. For example, plate $2.5\text{-}3 \times 10^6$ U2OS cells in a 150 mm dish.
3. Incubate cells at 37 °C in 5% CO₂ overnight.



Electroporation

Table 1. Recommended samples for a gene editing electroporation experiment.

Sample	Explanation of sample
Edit-R Cas9 Nuclease protein NLS with Non-targeting control guide RNA	Negative control: Cas9 protein without targeting RNAs
Edit-R Cas9 Nuclease protein NLS with tracrRNA and gene-specific guide RNA	Gene editing: Cas9 protein programmed by RNAs for targeted double-stranded break in gene of interest or control gene
Untransfected	No treatment control: confirmation of cell viability



It is recommended to perform electroporation of gene-specific guide RNA in triplicate along with the controls listed in Table 1 for high confidence experimental results.

- Prepare 6-well plates by transferring 2 mL of pre-warmed culture medium to each well required for the number of samples and replicates in the experiment. Pre-incubate/equilibrate by placing at 37 °C in 5% CO₂ while preparing samples.
- Prepare crRNA and tracrRNA or synthetic sgRNA samples for electroporation.

For crRNA and tracrRNA:

 - Prepare a 200 μM crRNA stock solution by adding the appropriate volume of nuclease-free, 10 mM Tris pH 7.4 buffer to crRNA. Verify the RNA concentration using UV spectrophotometry at 260 nm and adjust the volume if necessary to obtain 200 μM.
 - Prepare a 200 μM tracrRNA stock solution by adding the appropriate volume of nuclease-free, 10 mM Tris pH 7.4 buffer to tracrRNA. Verify the RNA concentration using UV spectrophotometry at 260 nm and adjust the volume if necessary to obtain 200 μM.
 - Prepare a 100 μM working solution of crRNA:tracrRNA by combining equal volumes of 200 μM stock solutions (1:1 ratio). Mix gently.

For synthetic sgRNA:

 - Prepare a 100 μM synthetic sgRNA stock solution by adding the appropriate volume of nuclease-free, 10 mM Tris pH 7.4 buffer to the sgRNA. Verify the RNA concentration using UV spectrophotometry at 260 nm and adjust the volume if necessary to obtain 100 μM.
- In 1.7 mL tubes, prepare samples to be electroporated. Combine 150 pmol of Cas9 protein with 3 μL (300 pmol) of crRNA:tracrRNA or synthetic sgRNA working solution for a final concentration of 1.5 μM and 3 μM, respectively. This creates the Cas9 ribonucleoprotein (RNP) complex.

Concentration	150 pmol	Total volume
20 μM	7.4 μL	10.4 μL
40 μM	3.7 μL	6.7 μL



Lower amounts of Cas9 protein with higher amounts of guide RNA can be electroporated. We found that using 100 pmol of Cas9 protein with 400-600 pmol guide RNA produced comparable gene editing as 150 pmol Cas9 protein with 300 pmol guide RNA; this ratio should be empirically determined for your cell line.

- Incubate at room temperature for 10-15 minutes.
- Collect 1×10^6 cells for each sample. Centrifuge at $\sim 500 \times g$ for 1 minute at room temperature.
- Aspirate medium from the cell pellet and resuspend in 100 μL of appropriate electroporation buffer.



Do not leave cells resuspended in electroporation buffer for more than 15 minutes as this can negatively affect cell viability.

- Transfer resuspended cells to the 1.7 mL tube containing Cas9 RNP complex. Gently mix components and transfer the entire volume to an electroporation cuvette. Sample should cover the bottom of the cuvette; tap to remove any air bubbles.
- Electroporate sample with program X-001.
- Use a transfer pipette to gently layer pre-incubated medium on top of electroporated cells from one well of a 6-well plate. Gently aspirate cells from the bottom of the cuvette and transfer to the well.
- Repeat steps 8-12 for remaining Cas9 protein gene editing samples.
- Incubate cells at 37 °C in 5% CO₂ for a total of 48 to 72 hours after electroporation before proceeding with gene knockout analysis.

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