

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

Successful electroporation of Edit-R Cas9 Nuclease mRNA 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 engineering can be ordered at dharmacon.com

- Edit-R Cas9 Nuclease mRNA, 20 µg (1 µg/µL; Cat #CAS11195, #CAS11859, or #CAS11860)
- Synthetic targeting guide RNA (choose one):
 1. Edit-R synthetic crRNA and tracrRNA oligos:
 - a. crRNA, [pre-designed for your gene of interest](#) in a variety of sizes, or designed and ordered using the [Dharmacon CRISPR Design Tool](#)
 - b. tracrRNA, 5, 20 or 50 nmol (Cat #U-002005-XX)

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



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 Design Tool](#)

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
- Phosphate-buffered saline (PBS)
- Assay(s) for detecting gene engineering events in a cell population
- 10 mM Tris pH 7.4 nuclease-free buffer (Tris buffer) solution (Dharmacon, Cat #B-006000-100)

General protocol for electroporation of Edit-R Cas9 Nuclease mRNA and synthetic guide RNA:

The following example provides a general protocol using electroporation to deliver Edit-R Cas9 Nuclease mRNA and guide RNAs 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 K-562 cells (2×10^6) 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. Count cells using a hemocytometer or other automated method.
2. Plate cells to achieve 70-80% confluence the next day. For example, plate 8×10^5 K-562 cells in a 150 mm dish.



Cell densities greater than 80% may reduce electroporation efficiency.

3. Incubate cells at 37 °C in 5% CO₂ overnight.

Table 1. Recommended samples for a gene engineering electroporation experiment

| Sample | Purpose |
|--|---|
| Edit-R Cas9 Nuclease mRNA with Non-targeting control guide RNA | Negative control: Cas9 mRNA without targeting guide RNAs |
| Edit-R Cas9 Nuclease mRNA with gene-specific guide RNA | Gene engineering sample: Cas9 nuclease programmed by guide RNAs for targeted double-stranded break in gene of interest |
| Untransfected | No treatment control sample: confirmation of cell viability |



It is recommended to perform electroporation of 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 appropriate culture medium to the number of wells required for each sample in the experiment. Pre-incubate/equilibrate by placing at 37 °C in 5% CO₂ while preparing samples.
- Prepare guide RNA samples for electroporation.
For crRNA and tracrRNA:
 - Prepare a 200 μM crRNA stock solution by adding the appropriate volume of Tris 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 Tris 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 Tris buffer to the sgRNA. Verify the RNA concentration using UV spectrophotometry at 260 nm and adjust the volume if necessary to obtain 100 μM.
- Prepare each sample to be electroporated in a 1.7 mL microcentrifuge tube by mixing 5 μg Cas9 mRNA (5 μL) with 5.4 μL of the 100 μM crRNA:tracrRNA or synthetic sgRNA working solution. This will result in 5 μM of guide RNA in the final electroporation mixture.



Cas9 mRNA and synthetic guide RNA volume to be electroporated should not exceed 11 μL (or ~10% of cell resuspension volume).

- Collect 2×10^6 cells for each sample. Centrifuge at $\sim 500 \times g$ for 2 minutes at room temperature.
- Aspirate medium from the cell pellet, wash once with phosphate-buffered saline (PBS) by adding buffer to gently resuspend cells and centrifuging again, and resuspend in 100 μL of Lonza kit V 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 1.7 mL tube containing Cas9 mRNA and guide RNA. 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 T-016.
 - 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 6-11 for remaining samples.
13. Incubate cells at 37 °C in 5% CO₂ for a total of 48 to 72 hours after electroporation; proceed with gene knockout analysis.



When using Fluorescent Cas9 mRNA, we suggest enriching for positive fluorescent cells using FACS 8-24 hours after electroporation. Refer to the protocol for “Using Dharmacon Edit-R Fluorescent Cas9 mRNA for enrichment of transfected cells” for more information.