

Notices

Limitations of use

For research use only. Not for use in diagnostic procedures. Unless otherwise agreed to in writing, IDT does not intend these products to be used in clinical applications and does not warrant their fitness or suitability for any clinical diagnostic use. Purchaser is solely responsible for all decisions regarding the use of these products and any associated regulatory or legal obligations.

Safety data sheets pertaining to this product are available upon request.

Safety notices



Reminder symbols call attention to minor details that may be easily overlooked and compromise the procedure resulting in decreased assay performance.



Caution symbols denote critical steps in the procedure where risk of protocol failure or damage to the product itself could occur if not carefully observed.



Stop symbols indicate where this procedure may be safely suspended and resumed at a later time without risk of compromised assay performance. Make note of these steps and plan your workflow accordingly.

Revision history

Version	Release date	Description of changes
New Document	October 2026	New protocol

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Technical support

Visit <https://www.idtdna.com/pages/support> for a helpful answers to frequently asked questions or contact us directly at applicationsupport@idtdna.com.

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Overview

Intended use

This protocol describes delivery of a Base Editor mRNA–sgRNA complex into HEK-293 cells using electroporation with the Lonza Nucleofector System.

Product/Kit Name/Part numbers

Materials Supplied		
Description	Part Number	Quantity
<i>Alt-R Base Editing Kit</i>	10038281	6 x 20µg (each ABE8e-based variant)
<i>ABE8e nCas9 mRNA, RG</i>	10037062, 10037063, 10037064	20µg, 100µg, 1mg
<i>ABE8e nSpG mRNA</i>	10037065, 10037066, 10037067,	20µg, 100µg, 1mg
<i>ABE8e nSPRY mRNA, RG</i>	10037068, 10037069, 100370670	20µg, 100µg, 1mg
<i>ABE8e-V106W nCas9 mRNA</i>	10037071, 10037072, 10037073	20µg, 100µg, 1mg
<i>ABE8e-V106W nSpG mRNA</i>	10037074, 10037075, 10037076	20µg, 100µg, 1mg
<i>ABE8e-V106W nSPRY mRNA, RG</i>	10037077, 10037078, 10037079	20µg, 100µg, 1mg



Materials and equipment required but not supplied. Use of other materials has not been tested by IDT.

Materials Required, but not Supplied		
Description	Supplier	Part Number
Alt-R™ CRISPR-Cas9 sgRNA or sgRNAXT	<i>IDT</i>	IDT predesigned and custom gRNA
Nuclease-Free IDTE Buffer, pH 7.5 (10 x 2 mL IDTE)	<i>IDT</i>	11-01-02-02
Nuclease-free water	<i>IDT</i>	11-04-02-01
Fetal Bovine Serum (FBS)	<i>N/A</i>	Any general lab supplier
Dulbecco's Modified Eagle's Medium (DMEM)	<i>ATTC</i>	30-2002 (or a general lab supplier)
TrypLE Express/Trypsin	<i>Gibco</i>	12604013 (or a general lab supplier)
1X Phosphate buffered saline (PBS)	<i>Gibco</i>	12604013 (or a general lab supplier)
SF Cell Line 96-well Nucleofector Kit	<i>Lonza</i>	V4SC-2096
4D-Nucleofector System	<i>Lonza</i>	AAF-1002B with AAF-1002X
96-well Shuttle System	<i>Lonza</i>	AAM-1001S
1 mM sodium citrate, pH 6.4	<i>Teknova</i>	S8513
96-well V-bottom plate	<i>Eppendorf</i>	03-14-01-06 (or general lab supplier)
96-well tissue culture plate	<i>Corning</i>	3596 (or a general lab supplier)

Before getting started

Important precautions

- Read through the entire protocol before starting.
- Use low-passage healthy cells.
- Subculture cells for at least 2–3 days before electroporation and replace medium the day before electroporation.
- Maintain cell confluency between 70–85%.
- Wash cells following trypsinization to reduce RNase exposure.

Protocol

Prepare CRISPR Reagents

1. Resuspend sgRNA in Nuclease-Free IDTE Buffer, pH 7.5 (part #11-01-02-02) to a concentration of 100 μM .

Note: If further assistance is needed, use the IDT Resuspension Calculator at www.idtdna.com/SciTools

sgRNA quantity (nmole):	Resuspension Volume (μL):	Concentration (μM):
2	20	100
10	100	100
50	500	100
100	1000	100

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2. Dilute resuspended sgRNA and base editor mRNA as described below to generate working concentrations:

Component	Stock Concentration	Working Concentration	Diluent
Base Editor mRNA	1 mg/mL	625 ng/ μ L	1 mM sodium citrate, pH 6.4 (Teknova, part # S8513)
sgRNA	100 μ M	25 μ M	Nuclease-Free IDTE Buffer, pH 7.5 (IDT, part #11-01-02-02)

Prepare mRNA-sgRNA complexes

3. For each well that is to be electroporated, combine the following components to a V-bottom plate to make the mRNA-sgRNA complex. The final volume is 5 μ L.

Component	Working Concentration	Amount	Final concentration in Nucleofection reaction
Base Editor mRNA	625 ng/ μ L	2 μ L	1 μ g
sgRNA	25 μ M	2 μ L	2 μ M
PBS	1X	1 μ L	-

Note: Keep reagents on ice *until* delivery into cells.

Transfect Cells by Nucleofection

The following outlines the delivery of mRNA-sgRNA reagent mix into HEK-293 cells. Cell density and electroporation conditions should be optimized for the cell type and application.

1. Prepare 96-well plate to receive cells following the nucleofection by adding 175 μ L of cell culture media per well and incubate at 37°C, 5% CO₂ until nucleofection is complete.

Note: IDT recommends dividing each nucleofection reaction into three replicate wells.

2. Warm an additional 80 μL of cell media at 37°C per nucleofection reaction.
3. Dissociate and harvest cells using trypsin or a cell dissociation buffer according to standard protocols.
4. Resuspend cell pellet in Nucleofection Solution SF.

Notes: Prior to first use, add entire Supplement to Nucleofection Solution SF, according to the manufacturer's recommendations. Each nucleofection reaction requires 200,000 cells per 20 μL .

5. To each well of the 96-well V-bottom plate containing 5 μL of the mRNA-sgRNA mixture, add 20 μL of cells resuspended in Nucleofection Solution SF. The new total volume is 25 μL . Gently mix the solution by slowly pipetting up and down 8-10 times.
6. From the 25 μL volume, transfer 20 μL of this mixture into each well of a 96-well Nucleocuvette plate. Gently tap the plate to remove any air bubbles.

Note: The concentration should be 2 μM sgRNA and 1 μg of base editor mRNA per well.

7. Electroporate using the desired method. For HEK-293 cells, we recommend pulse code DS-150.
8. Following electroporation, add 80 μL of the pre-warmed cell culture media to each well. Gently pipette to mix. The final volume is 100 μL .
9. Transfer 25 μL of transfected cells to each well of the 96-well plate containing 175 μL of cell culture media (as described in Step 1). Incubate cells at 37°C , 5% CO_2 for 72 hr prior to use in downstream applications or extraction of genomic DNA.

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