

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	July 17, 2026	New Protocol

Contents

Revision History.....	2
Overview	4
Required materials.....	5
Before getting started	6
Protocol	7

Technical support

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

www.idtdna.com | applicationsupport@idtdna.com

MasterControl Document Number

For research use only. Not for use in diagnostic procedures.

Page 3 of 9

Overview

Description

This protocol describes the delivery of a CRISPR-Cas9 PE mRNA, pegRNA and sgRNA into HEK-293 cells using electroporation with a Nucleofector system (Lonza)

Product/Kit Name/Part numbers

Materials Supplied		
Description	Part Number	Quantity
<i>PE Ultra mRNA, RG, 20 µg</i>	<i>10037049</i>	<i>20 µg</i>
<i>PE Ultra mRNA, RG, 100 µg</i>	<i>10037050</i>	<i>100 µg</i>
<i>PE Ultra mRNA, RG, 1 mg</i>	<i>10037061</i>	<i>1 mg</i>

 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
pegRNA	IDT	www.IDTDNA.com/PrimeEditing
Alt-R™ CRISPR-Cas9 sgRNA	IDT	www.IDTDNA.com/gRNA
SF Cell Line 96-well Nucleofector Kit	Lonza	V4SC-2096
4D-Nucleofector System	Lonza	AAF-1002B with AAF-1002X
96-well Shuttle System	Lonza	AAM-1001S
Dulbecco's Modified Eagle's Medium (DMEM)	ATTC	30-2002
1 M Tris-HCl, pH 7.5 (molecular biology grade)	IDT	11-01-02-02
Nuclease-free water	IDT	11-04-02-01
Fetal bovine serum (FBS)	General laboratory supplier	
Trypsin	General laboratory supplier	
1X Phosphate buffered saline (PBS)	General laboratory supplier	
1 mM sodium citrate, pH 6.4	General laboratory supplier	
96-well V-bottom plate	General laboratory supplier	
96-well tissue culture plate	General laboratory supplier	

Before getting started

Cell Culture Considerations

- Do not use freshly thawed cells for electroporation. Passage cells at least once prior to use.
- Use cells with lowest passage number possible (< 20 passages).
- Subculture cells for a minimum of 2-3 days prior to electroporation, replacing media the day before electroporation. Confirm normal, healthy cell morphology prior to use.
- Change cell media every 2-3 days while maintaining the cell line, making sure to include any necessary selection agents for stably transfected lines.
- Maintain cell culture at $\leq 90\%$ confluency. The highest electroporation efficiency is observed between 70 to 85% confluency.
- Wash cells in PBS following trypsinization, as both trypsin and FBS may contain RNase activity that quickly degrade RNA-based CRISPR components. Alternatively, consider using enzyme-free dissociation reagents in place of trypsin.

Protocol

Prepare CRISPR reagents

1. Resuspend pegRNA and sgRNA* in Nuclease-Free IDTE Buffer, pH 7.5 to a concentration of 100 μ M.

Note: For assistance, use the IDT Resuspension Calculator at www.idtdna.com/SciTools

2. Dilute resuspended sgRNA, pegRNA and Cas9 mRNA as described below to generate working concentrations:

Component	Stock concentration	Working concentration	Diluent
PE Ultra mRNA	1 mg/mL	625 ng/ μ L (0.625 mg/mL)	1 mM sodium citrate, pH 6.4
pegRNA	100 μ M	75 μ M	Nuclease-Free IDTE Buffer, pH 7.5
Alt-R™ Cas9 sgRNA	100 μ M	50 μ M	Nuclease-Free IDTE Buffer, pH 7.5

Prepare mRNA-pegRNA-sgRNA complex

For each well that is to be electroporated, combine the following components to a V-bottom plate to make the mRNA-pegRNA-sgRNA complex:

Component	Amount (μ L) of Working Concentration
PE Ultra mRNA	2
pegRNA	2
Alt-R™ Cas9 sgRNA [†]	1

[†]If no sgRNA is used, bring the reaction to volume (5 μ L total) with IDTE buffer pH 7.5

*The sgRNA referenced in this protocol is the nicking gRNA (ngRNA) used in the PE3 system. This secondary gRNA targets the non-edited DNA strand, which increases editing efficiency. For additional details, please refer to our FAQs.

Note: Keep reagents on ice until delivery into cells.

www.idtdna.com | applicationsupport@idtdna.com

MasterControl Document Number

For research use only. Not for use in diagnostic procedures.

Page 7 of 9

Transfect Cells by Nucleofection

The following outlines the delivery of mRNA-pegRNA-sgRNA reagent mix into HEK-293 cells at 2×10^5 cells in 20 μL per nucleofection reaction. Cell density and electroporation conditions should be optimized for the cell type and application.

Prepare cells as you would for a standard CRISPR Cas9 nucleofection experiment, making sure the cells are washed with PBS before nucleofection to remove any residual media/nucleases.

Note: Prior to first use, add entire Supplement to Nucleofection Solution SF, according to manufacturer recommendation.

1. Prepare 96-well plate to receive cells following the nucleofection by adding 175 μL of cell culture media per well and incubate at 37°C , 5% CO_2 until nucleofection is complete.
2. Warm an additional 80 μL of cell media at 37°C per nucleofection reaction.

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

3. Dissociate and harvest cells using trypsin or a cell dissociation buffer according to standard protocols.
4. Resuspend cell pellet in Nucleofection Solution SF.

Note: Each nucleofection reaction requires 2×10^5 cells per 20 μL (10^7 cells per mL).

5. Transfer 20 μL of cells resuspended in Nucleofection Solution SF to each well of the 96-well V-bottom plate containing mRNA-pegRNA-sgRNA mixture. Gently mix solution by slowly pipetting up and down 8-10 times.
6. Transfer 20 μL of cells and mRNA-pegRNA-sgRNA mixture to each well of a 96-well Nucleocuvette plate and gently tap plate to remove any air bubbles.

Note: Each nucleofection reaction is 20 μL and contains 6 μM pegRNA, 2 μM sgRNA and 1 μg of PE Ultra mRNA.

7. Electroporate using desired method. For HEK-293 cells, we recommend pulse code DS-150.
8. Following electroporation, add 80 μL of warm cell culture media to each well and gently pipette to mix.
9. Transfer 25 μL of transfected cells to each well of the 96-well plate that contains 175 μL of cell culture media from Step 1 above. Incubate cells at 37°C , 5% CO_2 for 72 hours prior to use in downstream applications or extraction of genomic DNA.

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.

© 2025 Integrated DNA Technologies, Inc. All rights reserved. Trademarks contained herein are the property of Integrated DNA Technologies, Inc. or their respective owners. For specific trademark and licensing information, see <http://www.idtdna.com/trademarks>.