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 or online at https://www.idtdna.com/pages/support/safety-data-sheets.

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.

Contents

Technical support

Visit https://www.idtdna.com/pages/support for helpful answers to frequently asked questions or contact our regional applications support teams directly at:

- North and South America (AMR): amr-applicationsupport@idtdna.com
- Europe, the Middle East, and Africa (EMEA): emea-applicationsupport@idtdna.com
- Asia-Pacific (APAC): apac-applicationsupport@idtdna.com

Overview

Description

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

S.p. Cas9 mRNA, RG & *Spy*Fi™ Cas9 mRNA, RG

S.p. Cas9 mRNA and *Spy*Fi Cas9 mRNA are provided in 1mM sodium citrate, pH 6.4 at 1 mg/mL. Recommended storage is at -80ºC.

Materials Supplied		
Description	**Part Number**	**Quantity (Amount, concentration, volume)**
S.p. Cas9 mRNA, RG, 20 µg	10030008	20 µg, 1 mg/mL, 20 µL
S.p. Cas9 mRNA, RG, 100 µg	10030004	100 µg, 1 mg/mL, 100 µL
S.p. Cas9 mRNA, RG, 1 mg	10030005	1 mg, 1 mg/mL, 1 mL
*Spy*Fi™ Cas9 mRNA, RG, 20 µg	10030009	20 µg, 1 mg/mL, 20 µL
*Spy*Fi™ Cas9 mRNA, RG, 100 µg	10030006	100 µg, 1 mg/mL, 100 µL
*Spy*Fi™Cas9 mRNA, RG, 1 mg	10030007	1 mg, 1 mg/mL, 1 mL

Required Materials

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	IDT	IDT predesigned and custom gRNA
Nuclease-free IDTE Buffer, pH 7.5	IDT	11-01-02-02
Nuclease-free water	IDT	11-04-02-01
Dulbecco's Modified Eagle Media (DMEM)	ATCC	30-2002
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
1 mM sodium citrate, pH 6.4	General laboratory supplier	
Fetal Bovine Serum (FBS)	General laboratory supplier	
Phosphate Buffered Saline, 1X	General laboratory supplier	
Trypsin, 1X	General laboratory supplier	
Enzyme-free Cell Dissociation Buffer (Optional)	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 ≤ 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 sgRNA in Nuclease-Free IDTE Buffer, pH 7.5 to a concentration of 100 µM.

Note: For assistance, use the IDT Resuspension Calculator.

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

Component	Stock concentration	Working concentration	Diluent
Alt-R™ Cas9 sgRNA	100 µM	30 µM	Nuclease-Free IDTE Buffer, pH 7.5
S.p. Cas9 mRNA or *Spy*Fi™ Cas9 mRNA	1 mg/mL	625 ng/µL (0.625 mg/mL)	1 mM sodium citrate, pH 6.4

Prepare mRNA-sgRNA complex

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

Component	Amount (µL) of Working Concentration
Alt-R™ Cas9 sgRNA	3
S.p. Cas9 mRNA or *Spy*Fi™ Cas9 mRNA	2
Total volume	**5**

Note: Keep reagents on ice until delivery into cells.

Transfect Cells by Nucleofection

The following outlines the delivery of mRNA-sgRNA reagent mix into HEK293 cells at 2 x 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 that 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 a 96-well plate to receive cells following nucleofection by adding 175 µL of cell culture media per well and incubate at 37ºC, 5% CO_2 until nucleofection is complete.
2. Warm an additional 80 µL of cell media at 37ºC 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 x 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-sgRNA mixture. Gently mix solution by slowly pipetting up and down 8–10 times.
6. Transfer 20 µL of cells and mRNA-sgRNA mixture to each well of a 96-well Nucleocuvette plate and gently tap the plate to remove any air bubbles.

Note: Each nucleofection reaction is 20 µL and contains 2 µM sgRNA and 1 µg Cas9 mRNA.

7. Electroporate using a desired method. For HEK293 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 37ºC, 5% CO_2 for 72 hr prior to use in downstream applications or extraction of genomic DNA.