ICAFectin®-Cas9:sgRNA Reagent for ribonulceoprotein transfection in primary and stem cells

Important note for transfection

- The Nanotaxi®-Cas9:sgRNA/Cas9:sgRNA complex must be prepared in medium that does not contain serum (Opti MEM is recommended.
- Do not add serum and antibiotics during the formation of the Nanotaxi®-Cas9:sgRNA/Cas9:sgRNA complexes.
- Cells are transfected during 3 hours in a medium that does not contain serum.
- Storage of Nanotaxi®-Cas9:sgRNA at +4°C. No need to keep Nanotaxi®-Cas9:sgRNA/Cas9:sgRNA complexes on ice during tranfection.
- Use conditions described in *Table 1* for optimizing transfection efficiency.

Transfection of adherent cells

Forward transfection procedure

Use the following procedure to transfect adherent cells in a 24-well format.

Cell Preparation

One day before transfection, plate cells in 1 mL complete growth medium so that cells reach 70-80% confluence at the time of transfection (1 x 10^5 cells per mL depending on the cells).

Nanotaxi®-Cas9:sgRNA/Cas9:sgRNA complex preparation

All amounts and volumes are given to transfect one well in a 24-well format.

1. Twenty minutes before transfection, remove growth medium and add 0.35 mL fresh of medium **without serum** per well.

2. Dilute 0.5 µg of sgRNA and 1.0 µg of Cas9 in 25 µL of Opti-MEM I **without serum**. Mix gently.
*Note : **Do not use** the serum-free culture medium in which cells were grown.*

3. Incubate 10 minutes at room temperature

4. Vortex Nanotaxi®-Cas9:sgRNA before use. Dilute 1.3 µL of Nanotaxi®-Cas9:sgRNA in 25 µL of Opti-MEM **without serum**. Mix gently.
*Note : **Do not use** the serum-free culture medium in which cells were grown.*

5. Add the diluted Nanotaxi®-Cas9:sgRNA to the diluted Cas9:sgRNA by pipetting up and down five times and briefly vortexing.

6. Mix gently and incubate 15 minutes at room temperature to allow formation of Nanotaxi®-Cas9:sgRNA/Cas9:sgRNA complexes

7. Add the entire volume of complexes drop-wise to each well containing cells. Swirl the plate to ensure the homogeneous distribution of complexes.

8. Incubate cells with transfection complexes under their normal condition growth. After 3 hours of incubation, medium is changed with fresh medium with serum.

All conditions to be tested with Nanotaxi® -Cas9:sgRNA:

Cas9 (µg)	sgRNA (µg)	Nanotaxi® - Cas9:sgRNA (µL)
1.0	0.5	1.3 µl
0.5	0.2	2.0 µl

Table 1 : *Conditions to be tested for optimization*