Protocol ● CRISPR-Fectin™Transfection Reagent ● Catalog Nos. EF015/EF016

For efficient transfection of Cas9-sgRNA complex into mammalian cells

Description

CRISPR-Fectin™Transfection Reagent is a proprietary lipid-based formulation that forms a complex with Cas9-sgRNA ribonucleoprotein and transports the complex into mammalian cells. CRISPR-Fectin™ has been proven to work in a wide range of commonly used cell lines. It is optimized for efficient and simple delivery of nucleic acids even in the presence of serum. CRISPR-Fectin™ provides the following advantages:

- Superior transfection efficiency for a broad range of cell lines compared with commonly used transfection reagents, such as Lipofectamine®2000 and Lipofectamine™ CRISPRMAX.
- Lowcytotoxicity.
- Does not require removal of serum or culture medium.
- Does not require washing or changing of medium aftertransfection.
- Transfection of CRISPR-Cas9 ribonucleoprotein for genome editing

Contents and storage

Each vial contains 1 ml of sterile CRISPR-Fectin™ transfection reagent.

CRISPR-Fectin™ is shipped with ambient temperature. Store the reagent at 4-8°C with the cap tightly closed. The reagent is stable for at least 12 months when stored at 4-8°C.

Quality control

Every lot of CRISPR-Fectin™ is tested by transfecting subconfluent HEK-293 cells with the GeneHero™ Cas9 nuclease (catalog number: GE001/GE002) complexed with sgRNA targeting HUWE gene. T7E1 assay is used to verify the insertions and deletions generated by non-homologous end joining (NHEJ) activity. Please refer the COA for details.

Protocol for transfection

Materials:

- CRISPR-Fectin™ transfection reagent
- Opti-MEM® I Reduced Serum Medium (Life Technologies**.** Catalog number: 31985-088).
- Cas9 Nuclease protein with NLS, sgRNA
- Rnase-free tips, tubes, etc.

Procedure:

Day 0. Seed cells

– If the cells are from a recent liquid nitrogen stock, passage the cells at least 2 times before transfection.

– The day before transfection, trypsinize and count the cells. Adjust the cell density and media volume according to the table below. Do not include antibiotics.

	6-well	24-well	96-well
Cell number per well	around 6 x 10^5 cells	around 1 x 10^5 cells	around 2.5 x 10^4 cells
Volume of media per well	2 ml	0.5 ml	100 µl

Day 1.

– The number of cells plated in each well should be about 30%~50% confluence on the day of transfection.

Cas9-sgRNA RNP preparation

1. Thaw Cas9 protein with NLS sequence and sgRNA on ice. Dilute Cas9 protein using suitable buffer as needed. Dilute sgRNA using nuclease-free water.
2. For each well, mix sgRNA, Cas9 Nuclease and Opti-MEM™I Reduced Serum Medium according to the table below. Mix well using pipette, reduce bubbles during pipetting.

	6-well	24-well	96-well
sgRNA	32.5 pmol	6.5 pmol	1.3 pmol
Cas9 Nuclease	4000 ng (25 pmol)	800 ng (5 pmol)	160 ng (1 pmol)
Opti-MEM™ I Medium	125 µl	25 µl	5 µl

3. Incubate at room temperature for 5 min to assemble the RNP complexes.

Transfect the RNP complex

4. Dilute CRISPR-Fectin™ transfection reagent in Opti-MEM™ I Medium according to the table below. Mix well.

	6-well	24-well	96-well
CRISPR-Fectin™	7.5 µl	1.5 µl	0.3 µl
Opti-MEM™ I Medium	125 µl	25 µl	5 µl

5. Incubate the CRISPR-Fectin Max™ transfection reagent in Opti-MEM™ I Medium at room temperature for 1 minute.
6. Add the diluted CRISPR-Fectin Max™ transfection reagent to the Cas9-sgRNA RNP mixture. Mix well by pipetting.
7. Incubate the mixture of RNP and transfection reagent at room temperature for 15 to 20 min, do not exceed 30 min.
8. Add the mixture to the cells according to the table and mix gently by rocking the plate back and forth.

	6-well	24-well	96-well
RNP/CRISPR-Fectin™ mixture	250 µl	50 µl	10 µl

9. Incubate the cells at 37°C in a CO_2 incubator for 2-3 days until they are ready to be assayed.