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

For efficient transfection of nucleic acids 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 animal 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.
- Low cytotoxicity.
- Does not require removal of serum or culture medium.
- Does not require washing or changing of medium after transfection.
- 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) complexed with synthetic sgRNA targeting HUWE gene. T7E1 assay is used to verify the insertions and deletions generated by non-homologous end joining (NHEJ) activity.

Before you start

Condition of cells

Always use high-quality cells that are well maintained and routinely authenticated which includes testing for bacteria, fungi, or Mycoplasma contamination. If the cells are from a recent liquid nitrogen stock, passage the cells at least 2 times before transfection.

Protocol for transient transfection

Materials:

- CRISPR-Fectin™ transfection reagent
- Opti-MEM® I Reduced Serum Medium (Life Technologies. Catalog number: 31985-088).

- Prepare 300 nM sgRNA by diluting the stock with nuclease-free water on ice.
- Prepare 150 ng/µl Cas9 nuclease (~900 nM) using dilution buffer on ice.
- Assemble the reaction at room temperature in the following order:

Component	Volume	Final Concentration
Nuclease-free water	20 µl	
10X Reaction Buffer	3 µl	

300 nM sgRNA	3 µl	30 nM
900 nM Cas9 Nuclease	1 µl	30 nM
Reaction Volume	**27 µl**	
Pipette to mix and incubate for 10 min at 25 ºC		
30 nM DNA substrate	3 µl	3 nM
Total reaction volume	**30 µl**	

- Mix thoroughly and incubate at 37 ºC for 15 min.
- Add 1 µl of Proteinase K to the reaction, mix thoroughly
- Incubate at room temperature for 10 min.
- Analyze the digestion by agarose gel electrophoresis or Fragment Analyzer.