Intracellular Protein Delivery with ProtiFect STAR™

Background

ProtiFect STAR™ is a highly efficient protein delivery reagent, able to deliver a broad range of proteins with various molecular weights and isoelectric points to the cytosol. The intracellular delivery mechanism of ProtiFect STAR™ is through membrane fusion, bypassing the typical endocytic pathway. ProtiFect STAR™ is able to deliver Cas9 ribonucleoprotein for efficient gene editing applications.

ProtiFect STAR™ / Protein Complex Formation

ProtiFect STAR™ is supplied in anhydrous ethanol, 10 mg/mL.

1. Dilute with HEPES buffer (20 mM, pH 7.4), (5 µL ProtiFect STAR™ in ethanol to 45 µL HEPES buffer), to make a 1 mg/mL ProtiFect STAR™ solution.
2. Prepare the ProtiFect STAR™ protein complex by diluting 2 µL of 1 mg/mL ProtiFect STAR™ with 28 µL HEPES buffer. Combine the 30 µL of diluted ProtiFect STAR™ solution with 20 µL of protein solution (1 µL of 1 mg/mL protein, e.g., Cas9/RNP, with 19 µL HEPES buffer) and incubate for 1 minute.
3. Add ProtiFect STAR™ protein complex solution to 450 µL serum-free DMEM or Leibovitz's L-15 medium and apply to cells.

Note: The ratio of ProtiFect STAR™ to protein in step 3 is given as a recommendation for Cas9/RNP, it may be necessary to optimize the ratio (ProtiFect STAR™ concentration of 4-8 µg/mL, and protein concentration of 2-8 µg/mL) to achieve maximum efficiency for intracellular delivery of other proteins.

Delivery of Cas9/RNP for *In Vitro* CRISPR/Cas9 Genome Editing

1. Seed cells in 24-well plates at 1 × 105 cells/well and culture overnight.
2. Prepare ProtiFect STAR™ Cas9/RNP complexes as per protocol. The recommended concentration of Cas9 protein, sgRNA and ProtiFect STAR™ is 2, 1 and 4 µg/mL.
3. Remove cell culture medium and wash cells with PBS.
4. Add 500 µL ProtiFect STAR™ Cas9/RNP complexes solution to per well and incubate at 37 ºC for 4 h.
5. Replace cells with fresh culture media and incubate for another 48 h before analysis.

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