ProteanFect™ TuffCell Transfection Kit

Quick Start Guide

What's in the Package

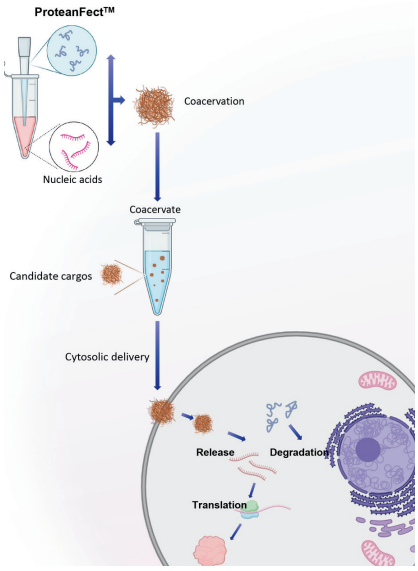
- 1x User Manual
- Reagent A, B, and C
- EGFP mRNA
- EGFF pDNA (with general kit)

Storage Requirement

- Entire Kit can be stored at -80°C
- Reagent A and C can be stored at 4°C

Quick Protocol: per well of a 96-well plate

1. **Transfection Complex Preparation**
   a. Mix Reagent A (40 µL) with mRNA (0.5 µg).
   b. Add Reagent B (1.4 µL) and mix with pipette (20-30x or vortex for 10 seconds).
2. **Cell Preparation**
   a. Suspension cells - Harvest cells by centrifugation at 300g for 5 min. Discard supernatant and wash once with Opti-MEM. Resuspend cells with Opti-MEM at $5x10^6$-$1x10^7$ cells/mL.
   b. Adherent cells - Maintain 50-80% cell confluence. Remove medium, wash cells once with Opti-MEM, add 20 µL of Opti-MEM. **Optional:** Harvest cells by trypsinization, then resuspend them in Opti-MEM at a concentration of $5×10^6$ - $1×10^7$ cells/mL for subsequent transfection.
3. **Transfection**
   a. Mix complex with cells: For suspension cells, mix 40 µL of transfection complex with 20 µL of cell suspension and gently pipet up and down 2-3 times. For adherent cells, apply directly to the cells.
   b. Incubate the cells with the transfection complex for 45-60 minutes in a cell culture incubator.
   c. Terminate the reaction by adding ≥200 uL of culture medium (10x cell suspension), then transfer the cells from the tube to the culture plate. For adherent cells, replenish with ≥200 µL of complete culture medium.
   d. Incubate transfected cells in culture medium and assess transfection efficiency after 5 to 48 hours, or at an appropriate time.



Additonal Notes

- Avoid including FBS in the transfection medium.
- The cell pellet may adhere to the tube walls. Gently remove the supernatant to minimize cell loss.

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