ProteanFect™ TuffCell Transfection Kit

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

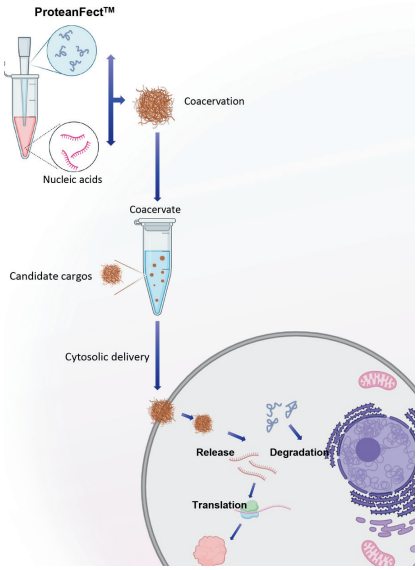
a. Mix Reagent A (40 µL) with mRNA (0.5 µg).

b. Add Reagent B (1.4 µL) and mix with pipette (20-30x or vortex for 10seconds).

2. Cell Preparation

a. Suspension cells - Harvest cells by centrifugation at 300g for 5 min.Discard supernatant and wash once with Opti-MEM. Resuspendcells with Opti-MEM at 5x10-1x10 cells/mL.[67]

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 pipetup and down 2-3 times. For adherent cells, apply directly to thecells.

b. Incubate the cells with the transfection complex for 45-60 minutesin a cell culture incubator.

c. Terminate the reaction by adding > 200 uL of culture medium (10xcell suspension), then transfer the cells from the tube to the cultureplate. For adherent cells, replenish with ≥200 µL of complete culturemedium.

d. Incubate transfected cells in culture medium and assesstransfection efficiency after 5 to 48 hours, or at an appropriatetime.

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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