Gene transfection

Jiao Wang

Abstract

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Protocol

Step 1.

Seed cells in 24-well plates at a density of 1 × 10^5 cells/500 µl antibiotic-free medium. Then culture cells under 5% CO_2, in a 95% humidified atmosphere at 37℃, for 24 h.

Step 2.

After 24 h, discard the medium and replace the fresh RPMI 1640 basal medium for transfection.

Step 3.

Dilute 0.5 µg pcDNA3-ATP11A and 0.5 µg pcDNA3-TMEM30A plasmids with 20 µl RPMI 1640 basal medium, and gently mix five times.

Step 4.

Gently vortex and mix the transfection reagent, dilute 1 µl *Lipofectamine*™ 2000 (Gibco, USA) with 25 µl RPMI 1640 basal medium, gently breathe five times and allow to stand for 5 minutes at room temperature.

Step 5.

Mix the transfection reagent and plasmid dilution, gently aspirate five times to mix and incubate at room temperature for 20 minutes.

Step 6.

Dropwise add the transfection mixture of *Lipofectamine*™ 2000-RPMI 1640 medium (50 µl / well) to the cells, and gently mix.

Step 7.

Incubate the cells under 5% CO_2 in a 95% humidified atmosphere at 37℃ for 6 h. After 6 hours, replace the medium with fresh.