Protocol for STO Cell Transfection by FuGENE HD

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Abstract

Protocol for Transfection Mouse Embryonic Stem Cells.

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Protocol

Step 1.

Cell plating

STO cells were seeded the day before transfection with the density 15,000 cells per well in 100 µl complete growth medium DMEM+10% Fetal Bovine Serum.

Step 2.
Prepare 0.02µg/µl pCMVβ plasmid DNA solution in OptiMEM®.

Step 3.

Add 6µl of reagent to 100 µl of OptiMEM® /DNA solution.

Step 4.
Mix carefully by pipetting (10-15 times).

Step 5.
Incubate 5 min at room temperature.

Step 6.

Add 5µl complex per well to the cells, and mix thoroughly.

Step 7.
Place the cells into CO2 incubator for 26-28 hours.

Step 8.
Remove the medium from the well and wash the cells once with 100µl per well PBS.

Step 9.
Fix the cells in the well with 50µl solution of 4% formaldehyde in PBS for 5min at room temperature.

Step 10.
Wash each well twice with 100µl PBS.

Step 11.

Add 50µl per well of substrate/stain solution and incubate the plate overnight at 37°C.

Step 12.
Observe the cells under microscope and evaluate the proportion of blue (β-gal-positive) cells.