

Protocol for STO Cell Transfection by FuGENE HD Version 2

Misha Gurevich, V. Katerov

Abstract

Protocol for Transfection Mouse Embryonic Stem Cells.

Citation: Misha Gurevich, V. Katerov Protocol for STO Cell Transfection by FuGENE HD. [protocols.io](https://doi.org/10.17504/protocols.io.e8sbhwe)

[dx.doi.org/10.17504/protocols.io.e8sbhwe](https://doi.org/10.17504/protocols.io.e8sbhwe)

Published: 23 Jun 2016

Protocol

Cell plating

Step 1.

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

Complex preparation (per 20 wells)

Step 2.

Prepare 0.02 μ g/ μ l pCMV β plasmid DNA solution in OptiMEM®.

📌 NOTES

Misha Gurevich 23 Jun 2016

Tissue culture 96-round bottom well plates were used for complex preparation.

Complex preparation (per 20 wells)

Step 3.

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

Complex preparation (per 20 wells)

Step 4.

Mix carefully by pipetting (10-15 times).

Complex preparation (per 20 wells)

Step 5.

Incubate 5 min at room temperature.

🕒 DURATION

00:05:00

Complex preparation (per 20 wells)

Step 6.

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

NOTES

Misha Gurevich 23 Jun 2016

Optimal ratio reagent/DNA may vary in range 0.2µl/0.1µg to 0.35µl/0.1µg.

Incubation

Step 7.

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

DURATION

26:00:00

Detection of β-gal expression

Step 8.

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

Detection of β-gal expression

Step 9.

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

DURATION

00:05:00

Detection of β-gal expression

Step 10.

Wash each well with 100µl PBS. (1/2)

Detection of β-gal expression

Step 11.

Wash each well with 100µl PBS. (2/2)

Detection of β-gal expression

Step 12.

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

DURATION

16:00:00

Detection of β-gal expression

Step 13.

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