Protocol for transfection of H4, (ATCC®: HTB-148) Cells by FuGENE HD in 96 well plates.

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Abstract

This is a protocol for transfection of H4 (Human Brain Neuroglioma) in either 10% or 100% FBS using FuGENE HD.

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Protocol

Step 1.

Cell plating

H4 (neuroglioma) cells were seeded from 90-100% confluent culture the day before transfection with the density 7,000 cells/well in 100µl complete growth medium (DMEM + 10% Fetal Bovine Serum).

For transfection in 100% Fetal Bovine Serum the complete growth medium was replaced with Fetal Bovine Serum two hours before transfection.

Step 2.

Complex preparation (per 20 wells)

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

Step 3.
Prepare 0.02µg/µl pCMVβ DNA solution in OptiMEM® or sterile deionized water.

Step 4.
Add 6 µl of reagent to 100 µl of DNA solution.

Step 5.
Mix carefully by pipetting (15 times).

Step 6.
Incubate 10 min at room temperature.

Step 7.
Add 5 µl of complex per well to the cells, and mix thoroughly.

Step 8.

Incubation

Incubate transfected cells in CO_2 incubator for 48 hours.

Step 9.

Detection of β-gal expression

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

Step 10.
Fix the cells in the well with 50µl solution of 4% formaldehyde in PBS for 5min at room temperature.

Step 11.
Wash each well twice with 100µl PBS.

Step 12.
Add 50µl per well of substrate/stain solution and incubate the plate overnight at 37°C.

Step 13.
Observe the cells under microscope and evaluate the proportion of blue (β-gal-positive) cells.