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Transfection of HT22 cells with Lipofectamine LTX V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract

Transfection of HT22 cells



Day 0

- 1 Plate HT22 cells in 6 well plate: 250K per well
If you want to perform transfection next day plate 500K.

Day 1

- 2 Check cells -> less than ~60-70% confluent wait another day

Day 3

- 3 Dilute the optimized amount of plasmid DNA in 500 μ l Opti-MEMI Reduced Serum Medium (no A/A, no FCS). Mix thoroughly.
- 4 Mix PLUS™ Reagent gently before use, add 2.5uL volume of PLUS™ Reagent directly to the diluted DNA. Mix gently and incubate for 5 minutes at room temperature.
- 5 Mix Lipofectamine LTX gently before use, and add 5uL directly to the diluted DNA. Mix thoroughly.
- 6 Incubate for 30 minutes at room temperature (DNA-lipid complexes are stable for 6 hours at room temperature)
- 6.1 Meanwhile: Add 2mL of antibiotic-free medium containing 5% FCS
- 7 Add ~500 μ l DNA-lipid complex (step 4 above) dropwise to the well containing cells. Mix gently by rocking the plate back and forth.
- 8 Incubate the cells at 37°C in a CO2 incubator for 18–48 hours prior to testing for transgene expression.
- 9 Change medium after 4–6 hours to regular medium (with A/A and 10% FCS)