



Transfecting Plasmid DNA into Primary Mouse Neural Progenitor Cells Using Lipofectamine™ LTX Reagent

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Introduction

Lipofectamine LTX™ Reagent is a proprietary, animal-origin free formulation for the transfection of DNA into eukaryotic cells with low cytotoxicity. This reference provides a recommended procedure to transfect plasmid DNA into Primary Mouse Neural Progenitor Cells (mNPC) using Lipofectamine™ LTX Reagent (Cat. No. 15338-100).

Important Guidelines for Transfection

Follow these important guidelines when transfecting mNPC cells using Lipofectamine LTX™ Reagent:

- Maintain the same seeding conditions between experiments. Use low-passage cells; make sure cells are healthy and greater than 90% viable before transfection.
- NCP's grow as neurospheres, and can be adhered to culture plates and differentiated into neurons, astrocytes, and oligodendrocytes upon addition of serum and insulin-like growth factor.
- Transfection can be performed both in the presence or absence of serum. Test serum-free media for compatibility with Lipofectamine LTX™ Reagent.
- Using PLUS Reagent (Cat. No. 11514-015) enhances transfection performance in Primary Mouse Neural Progenitor Cells.
- We recommend Opti-MEM® I Reduced Serum Medium (Cat. No. 31985-062) to dilute the DNA and Lipofectamine™ LTX Reagent before complexing.
- Visit www.invitrogen.com/transfection or contact Technical Service for other specialized transfection protocols.
- Lipofectamine™ LTX Reagent performs well with vector-based RNAi experiments. For siRNA and Stealth™ RNAi transfections, we recommend Lipofectamine™ RNAiMAX (Cat. No. 13778-075). Go to www.invitrogen.com/RNAi or contact Technical Service for more information.

Materials Needed

Have the following reagents on hand before beginning:

- Primary Mouse Neural Progenitor (NCP) cells maintained in DMEM (GIBCO Cat. No. 11960-044) medium supplemented with 1% L-glutamine (GIBCO Cat. No. 25030), 1% penicillin-streptomycin (Cat. no. 15070-063), 1% dextrose, and epidermal growth factor. Grow cells at 37° C with 5% CO₂.
- Plasmid DNA of interest (100 ng/μl or higher)
- Lipofectamine™ LTX Reagent (store at +4°C until use), and PLUS™ Reagent (store at 4°C)
- Opti-MEM® I Reduced Serum Medium
- Appropriate tissue culture plates and supplies

Transfection of Primary Mouse Neural Progenitor Cells

Use this procedure to transfect plasmid DNA into Primary Mouse Neural Progenitor Cells in a **24-well format**. All amounts and volumes are given on a per well basis.

1. Begin transfection one or seven days after differentiation in 24-well plate.
2. The day of transfection, remove growth medium from cells and replace with 0.5 ml of complete growth medium without antibiotics.
3. For each well of cells to be transfected, dilute 0.25, 0.50, or 1.0 μg of DNA into 100 μl of Opti-MEM® I Reduced Serum Medium without serum.
4. Mix PLUS™ Reagent gently before use, then add 2.5 μl PLUS™ Reagent directly to the diluted DNA. Mix gently and incubate for 5-15 minutes at room temperature.
5. For each well of cells, add 0.1-2.50 μl of Lipofectamine™ LTX Reagent into the above diluted DNA solution, mix gently and incubate for 30 minutes at room temperature to form DNA-Lipofectamine™ LTX complexes.
6. After the 30 minute incubation, add 100 μl of the DNA-Lipofectamine™ LTX complexes directly to each well containing cells and mix gently by rocking the plate back and forth.
7. Complexes do not have to be removed following transfection. Incubate the cells at 37°C in a CO₂ incubator for 18-24 hours post-transfection before assaying for transgene expression.

Notes:

- Results are best for NPC's differentiated for 1 day (compared to 7-day differentiated).
- Combinations that gave best results:
 - 1.5 μl Lipofectamine LTX / 2.5 μl PLUS/ 1.0 μg plasmid
 - 2.5 μl Lipofectamine LTX/ 2.5 μl PLUS/ 500 ng plasmid

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