



Transfecting Plasmid DNA into E18 Primary Rat Hippocampal Neurons 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 E18 Primary Rat Hippocampal Neurons using Lipofectamine™ LTX Reagent (Cat. No. 15338-100).

Important Guidelines for Transfection

Follow these important guidelines when transfecting DNA into E18 Primary Rat Hippocampal Neurons using Lipofectamine™ LTX Reagent:

- The addition of antibiotics to media during transfection may result in cell death, and has not been tested for E18 Primary Rat Hippocampal Neurons. If you wish to use antibiotics during transfection, test your conditions thoroughly.
- Maintain the same seeding conditions between experiments. Use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection.
- Transfection can be performed both in the presence or absence of serum. Test serum-free media for compatibility with Lipofectamine™ LTX Reagent.
- 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 Support for other specialized transfection protocols (including cell-type specific advice on use of PLUS™ Reagent and antibiotics, and a protocol for vector-based RNAi).
- 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 Support for more information.

Materials Needed

- Live primary embryonic day 18 rat hippocampal tissue was obtained from Genlantis (San Diego, CA). Initial culture was maintained in NeuroPure™ Plating Medium supplied with the tissue. After the initial plating, cultured cells were maintained in Neurobasal™ A Medium (Cat. No. 10888) supplemented with 0.5 mM L-glutamine (Cat. No. 25030), and B-27 Serum-Free Supplement (Cat. No. 17504). 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).
- Opti-MEM® I Reduced Serum Medium
- Appropriate tissue culture plates and supplies

Transfecting E18 Primary Rat Hippocampal Neurons

Use this procedure to transfect plasmid DNA into E18 Primary Rat Hippocampal Neurons in a **24-well format** (for other formats, see **Scaling Up or Down Transfections**, below). All amounts and volumes are given on a per well basis.

1. Prepare isolated neurons as described by the manufacturer. Plate 2.5×10^4 – 5.0×10^4 cells on 24-well poly D-lysine coated tissue culture plates in 0.5 ml of NeuroPure™ Plating Medium. Three days after cell seeding, remove media and replace with 0.5 ml Neurobasal™ A medium. Cells are incubated for 24 hours prior to transfection. Cell density should be 50–80% confluent on the day of transfection.
2. For each well of cells to be transfected, dilute 0.25 μg of DNA into 100 μl of Opti-MEM® I Reduced Serum Medium without serum.
3. For each well of cells, 0.375–1.0 μl of Lipofectamine™ LTX into the above diluted DNA solution, mix gently and incubate for 25 minutes at room temperature to form DNA-Lipofectamine™ LTX complexes.
4. Remove growth medium from cells and replace with 0.5 ml of complete growth medium. 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.
5. 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.

Scaling Up or Down Transfections

To transfect E18 Primary Rat Hippocampal Neurons in different tissue culture formats, vary the amounts of Lipofectamine™ LTX Reagent, DNA, cells, and medium used in proportion to the relative surface area, as shown in the table (amounts given on a per well basis).

Culture vessel	Surface area per well ¹	Volume plating medium	Cells per well	Volume dilution medium ²	DNA	Lipofectamine™ LTX Reagent
96-well	0.3 cm ²	100 μl	1.0×10^4	20 μl	50 ng	0.075–0.2 μl
48-well	1 cm ²	200 μl	2.0×10^4	40 μl	100 ng	0.15–0.4 μl
24-well	2 cm ²	500 μl	5.0×10^4	100 μl	250 ng	0.375–1.0 μl
12-well	4 cm ²	1 ml	1.0×10^5	200 μl	0.5 μg	0.075–2.0 μl
6-well	10 cm ²	2 ml	2.5×10^5	500 μl	1.25 μg	1.5–4.0 μl

¹Surface area may vary depending on the manufacturer.

²If the volume of Lipofectamine™ LTX Reagent is too small to dispense accurately, and you cannot pool dilutions, predilute Lipofectamine™ LTX Reagent 10-fold in Opti-MEM® I Reduced Serum Medium, and dispense a 10-fold higher amount (should be at least 1.0 μl per well). Discard any unused diluted Lipofectamine™ LTX Reagent.

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