



Transfecting Plasmid DNA into BG01V 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 human embryonic stem cell (hESC) BG01V using Lipofectamine™ LTX Reagent (Cat. No. 15338-100).

Important Guidelines for Transfection

Follow these important guidelines when transfecting DNA into BG01V using Lipofectamine™ LTX Reagent:

- The addition of antibiotics to media during transfection may result in cell death, and has not been tested for BG01V cells. 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.
- Using PLUS™ Reagent (Cat. No. 11514-015) enhances transfection performance in BG01V 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 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

- BG01V cells adapted for feeder-free growth are maintained in MEF conditioned hESC media consisting of Advanced D-MEM/F-12 (Cat. No. 12634) supplemented with 2 nM L-glutamine (Cat. No. 25030), 20% Knockout™ Serum Replacement (Cat. No. 10828), 1X MEM Non-Essential Amino Acids Solution (Cat. No. 11140), 0.1 mM β-mercaptoethanol, and 4 ng/ml bFGF (Basic Fibroblast Growth Factor, human, Cat. No. PHG0024). 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 (if desired; store at 4°C)
- Opti-MEM® I Reduced Serum Medium
- Appropriate tissue culture plates and supplies

Transfecting BG01V Cells

Use this procedure to transfect plasmid DNA into BG01V cells 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. BG01V cells adapted for feeder-free growth are grown to near confluence in conditioned hESC media in a 60 mM plate. The day before transfection, dislodge cells with 1 μg/ml collagenase diluted in hESC media. Cells are used to seed two 24-well plates coated with Matrigel for cell adherence. Cell density should be 50–80% confluent on the day of transfection.
2. **Optional:** The day of transfection, remove growth medium from cells and replace with 0.5 ml complete growth medium.
3. For each well of cells to be transfected, dilute 1.0 μg of DNA into 100 μl of Opti-MEM® I Reduced Serum Medium without serum.
4. If using PLUS™ Reagent: Mix PLUS™ Reagent gently before use, then 1.0 μl PLUS™ Reagent (a 1:1 ratio to DNA) directly to the diluted DNA. Mix gently and incubate for 5-15 minutes at room temperature.
5. For each well of cells, dilute 2.5–4.5 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.
6. 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.
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.

Scaling Up or Down Transfections

To transfect BG01V cells in different tissue culture formats, vary the amounts of Lipofectamine™ LTX Reagent, DNA, cells, medium and PLUS™ Reagent 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	Volume dilution medium ²	DNA	Lipofectamine™ LTX Reagent	PLUS™ Reagent
96-well	0.3 cm ²	100 μl	20 μl	200 ng	0.5–0.9 μl	0.2 μl
48-well	1 cm ²	200 μl	40 μl	400 ng	1.0–1.8 μl	0.4 μl
24-well	2 cm ²	500 μl	100 μl	1 μg	2.5–4.5 μl	1.0 μl
12-well	4 cm ²	1 ml	200 μl	2 μg	5.0–9.0 μl	2.0 μl
6-well	10 cm ²	2 ml	500 μl	5.0 μg	12.5–22.5 μl	5.0 μl

¹Surface areas 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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