GeneCopoeia™
Expressway to Discovery

Protocol • EndoFectin™ Lenti Transfection Reagent • Catalog Nos. EFL1001-01/02

For co-transfecting lentiviral expression constructs and packaging plasmids into packaging cells and production of lentiviral particles

Description

EndoFectin™ Lenti Transfection Reagent is a proprietary cationic polymer formulation that forms a complex with nucleic acids and transports the complex into animal cells. EndoFectin Lenti has been specifically optimized for co-transfection of lentiviral expression constructs and packaging plasmids into GeneCopoeia 293Ta packaging cell line (GeneCopoeia Catalog Number CLv-PK-01) and production of lentiviral particles. It is optimized for efficient and simple delivery of nucleic acids even in the presence of serum. EndoFectin Lenti provides the following advantages:

- **Superior transfection efficiency**
- **High production of lentiviral particles in 293Ta cells**
- **Compatibility with serum-containing media**
- **Low cytotoxicity**
- **Easy to use**

Contents and storage

Each vial contains one 1 ml of sterile-filtered EndoFectin Lenti reagent.

EndoFectin Lenti is shipped at ambient temperature. Store the reagent at 4–8°C with the cap tightly closed. The reagent is stable for at least 12 months when stored at 4–8°C.

Quality control

Each lot of EndoFectin Lenti is tested by transfecting subconfluent HEK-293Ta cells with an eGFP-expressing plasmid (GeneCopoeia Catalog No. EX-EGFP-Lv01). Over 95% of cells expressed eGFP 16 hours post-transfection.

Before you start

Quality of plasmid
It is critical to use plasmid of the highest quality. Determine the DNA concentration by reading the absorption at 260 nm. DNA purity is measured by using the 260 nm / 280 nm ratio (the ratio should be in the range of 1.8 to 2.0). Check the plasmid integrity by agarose gel electrophoresis.

Condition of cells
Always use healthy cells that are well maintained and passaged regularly. Make sure the culture is free from bacteria, fungi, or *Mycoplasma* contamination. If the cells were from a recent liquid nitrogen stock, passage the cells at least 2 times before transfection.

Protocol for co-transfecting lentiviral expression constructs and packaging plasmids into 293Ta packaging cells

Please refer to the protocol found in the **Lenti-Pac™ FIV Expression Packaging Kit** (User's Manual # LV001) or **Lenti-Pac™ HIV Expression Packaging Kit** (User's Manual # LV002). Contact GeneCopoeia Technical Services to have a copy send to you (email: inquiry@genecopoeia.com or call 301-762-0888).

Protocol for transient transfection

1. **Plate cells**[1]

Two days before transfection, trypsinize and count the 293Ta cells (GeneCopoeia Cat# CLv-PK-01). Adjust the cell concentration and plate 293Ta cells in a cell-culture vessel with a total volume as suggested in Table 1. The number of cells plated in each well should be determined so that they are 70-80% confluent at the time of transfection.

2. Prepare DNA/EndoFectin complex

DNA, EndoFectin Lenti reagent, and diluents should be acclimated to room temperature prior to the following steps.

Dilute the required amount of DNA with Opti-MEM I. Refer to table 1 for suggested volumes. Dilute EndoFectin Lenti reagent also with Opti-MEM I. Use 3.0 µl of EndoFectin reagent per 1 µg of DNA[3].

Add the diluted EndoFectin Lenti reagent drop-wise to the DNA solution while gently vortexing the DNA-containing tubes. (**Note: Do not reverse the addition sequence**.) Use round-bottom polypropylene tubes such as Falcon® 5-ml or 14-ml tubes (BD) for larger volumes.

Incubate the mixture for 10–25 minutes at room temperature to allow the DNA-EndoFectin complex to form.

Table 1. Suggested starting conditions for transfection of 293Ta packaging cells.

Culture Vessel	Surface Area (cm^2)	Volume of medium	Total amount of DNA per well	DNA dilution Volume	Ratio of EndoFectin (µl) to DNA (µg)	EndoFectin dilution Volume
6-well plate (one well)	9.3	2.0 ml	0.5 - 1.4 µg	50 - 200 µl	3:1	50 - 200 µl
35-mm dish	7.5	2.0 ml	0.4 - 1.2 µg	50 - 200 µl	3:1	50 - 200 µl
10-cm dish	49.0	10 ml	2.5 - 7.5 µg	0.5 - 1 ml	3:1	0.5 - 1 ml
15-cm dish	143.0	25 ml	7.5 - 20 µg	0.5 - 2 ml	3:1	0.5 - 2 ml

3. Transfect packaging cells

Add the DNA-EndoFectin Lenti complex directly to each well and gently swirl the plates/dishes.

For transfection in the absence of serum, remove the normal growth medium and replace with serum-free D-MEM, then add the DNA-EndoFectin Lenti complex. Add ½ volume of 30% serum in D-MEM 3 hours after transfection.

Replace overnight culture medium within 16 hours post-transfection with fresh D-MEM medium supplemented with 2-5% heat-inactivated fetal bovine serum and antibiotics and continue incubation in the CO_2 incubator at 37°C.

4. Harvest lentivirus

Collect the pseudovirus-containing culture medium in sterile capped tubes 48 hours post transfection[4], and centrifuge tubes at 500×g for 10 minutes to get rid of cell debris. Following centrifugation, filter the supernatant through 0.45 µM polyethersulfone (PES) low protein-binding filters[5].

Special notes

1. Plating packaging cells 2 days pre-transfection significantly increases lentivirus titers compared to plating cells one day before transfection.

   Use heat-inactivated fetal bovine serum for lentivirus production. It can be purchased from other vendors, or prepared by incubating thawed serum for 30 minutes at 56°C with gentle shaking.

2. The DNA-EndoFectin complex must be formed in the absence of proteins even though the complex is able to transfect cells in the presence of 10% serum. Opti-MEM I is recommended for optimal transfection efficiency. Serum-free D-MEM can be used in place of Opti-MEM I but the transfection efficiency will be compromised.

3. The ratio of 3.0 µl of EndoFectin Lenti reagent per 1 µg of DNA is efficient for transfecting 293Ta cells. Increasing the ratio does not further improve transfection efficiency.

4. Peak virus production is normally achieved 24-48 hours post transfection. Alternatively, lentiviral-containing supernatants may be collected multiple times at 36, 48, and 60 hours post-transfection. Supernatants should be replaced with fresh D-MEM medium supplemented with 2-5% heat-inactivated fetal bovine serum and antibiotics.

5. Do not use nitrocellulose filters, since nitrocellulose is known to bind lentivirus and reduce lentiviral titers.

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