Cat # SL100478 Store at 4 °C

CalFectin™ Mammalian DNA Transfection Reagent

----- A Protocol for Lentivirus Production in 293T Cells

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

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This product is for laboratory research ONLY and not for diagnostic use

Introduction:

CalFectin™ Mammalian DNA Tranfection reagent is a pre-optimized and refined version of traditional calcium phosphate transfection reagent. With a new chemistry, CalFectin™ reagent contains much lower concentration of calcium, leading to much lower cytotoxicity With boosted transfection efficiency. In addition, CalFectin™ reagent is an effective method for the production of long-term stable and transient transfectants on most adherent cell lines. Compared with liposome and polymer based transfection reagents, CalFectin™ reagent shows distinguished features and much higher efficiency on HepG2, HEK293, CHO, Hela, MDCK, COS, 3T3, LNcap, C6, PC12 and primary cultured cells, etc. This protocol is for generating lentivirus with CalFectin™ reagent from 293T cells.

Procedures for Transfecting 293T Cells:

Cell Seeding (see Table 1):

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~90% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection.

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. We recommend using complete serum/antibiotics-containing medium as a starting point.

Table 1. A Guideline for Seeding Adherent Cells Prior to Transfection in Different Culture Formats

Culture Dishes	Surface Area (cm2)	Number of Cells to Seed
100 mm Dish	58	2.2 – 4.4 x 10^6
60 mm Dish	21	0.9 – 1.8 x 10^6
35 mm Dish	9.6	3.5 – 7.0 x 10^5
6-well Plate	9.6	4.0 – 8.0 x 10^5
12-well Plate	3.5	1.5 – 3.0 x 10^5
24-well Plate	1.9	0.8 – 1.6 x 10^5
48-well Plate	1.0	4.0 – 8.0 x 10^4
96-well Plate	0.3	1.2 – 2.4 x 10^4

Preparation of CalFectin™-DNA Complex and Transfection Procedures

The following protocol is given for transfection in 10 cm dish. For other culture formats, scale up or down per culture dish's surface. The optimal transfection conditions are given in the standard protocol described below.

- Cell confluency should be ~90 % at the day of transfection
- For each 10 cm dish, add 5.0 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
- For each dish, dilute total 10 µg of DNA (5 µg lenti-vector plasmid plus 5 µg lentivirus packaging mix) into 500 µL of serum-free DMEM with High Glucose. Vortex gently to mix.
- For each dish, dilute 30 µL of CalFectin™ reagent into 500 µL of serum-free DMEM with High Glucose. Vortex gently to mix.
  Note: Never use Opti-MEM to dilute DNA and CalFectin™ reagent because it will disrupt transfection complex.
- Add the diluted CalFectin™ Reagent immediately to the diluted DNA solution all at once. **(Important: do not mix the solutions in the reverse order !)**
- Vortex to mix the solution immediately followed by incubation of 10 minutes at room temperature to allow CalFectin™-DNA complexes to form.
  Note: Never keep the DNA/CalFectin™ complex longer than 20 minutes
- Add the 1000 µL CalFectin™/DNA complex dropwise onto the medium in each dish and homogenize the mixture by gently swirling the plate.
- Change medium 24 hr after transfection followed by harvesting lentivirus from supernatant 48 hr and 72 hr post transfection.

Storage: CalFectin™ Reagent is stable for up to 12 months at +4 °C after receipt