

CalFectin™ Mammalian DNA Transfection Reagent

----- A Protocol for Transfecting Mammalian 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 Transfection 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.

Procedures for Transfecting Adherent 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 ~70% 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 (cm ²)	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

For different cell types, the optimal ratio of CalFectin™ (µL):DNA (µg) varies from 2:1 to 3:1. We recommend the CalFectin™ (µL):DNA (µg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency without visible cytotoxicity. To ensure the optimal size of CalFectin™/DNA complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and CalFectin™ Reagent.

The following protocol is given for transfection in 24-well plates, refer to **Table 2** for transfection in other culture formats. The optimal transfection conditions for a majority of adherent cell lines, as well as a general starting point for optimization are given in the standard protocol described below.

- For each well, add 0.5 ml of complete medium with serum and antibiotics freshly 30-60 minutes before transfection.
- For each well, dilute 0.5 µg of DNA into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to the bottom of the tube.
 - Note:** Never use Opti-MEM to dilute CalFectin™ reagent and DNA, it will disrupt formation of transfection complex.
- Add 1.5 µl of CalFectin™ reagent immediately and directly into the 50 µl diluted DNA solution. Pipetting up and down 3-4 times to mix.
- Incubate for 10-15 minutes at room temperature to allow CalFectin™/DNA complexes to form.
 - Note:** Never keep the CalFectin™/DNA complex longer than 20 minutes.
- Add the 50 µl CalFectin™/ DNA mixture drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
- Remove CalFectin™/DNA complex-containing medium and replace with fresh complete serum/antibiotics containing medium 12-18 hours post transfection.
- Check transfection efficiency 24 to 48 hours post transfection.

Table 2. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Volume (ml)	Plasmid DNA (µg)	Diluent Volume (mL)	CalFectin™ Reagent (µL)
48 well plate	0.2	0.25	0.025	0.75
6-well plate	1	1	0.1	3
35 mm dish	1	1	0.1	3
60 mm dish	2.8	2.5	0.25	7.5
10 cm dish	5	3 - 4	0.5	9 - 12
T75 flask	8	9 - 18	0.75	27 - 54
250 ml flask	18	25 - 50	1.25	75 - 150

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