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PrimeFect™ DNA Transfection Reagent for Hela Cells

Cat. #: M0003-HC Size: 1 ml

Procedures for Transfecting Hela Cells:

Step I. 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 anti-biotics is freshly added to each well ~60 minutes before transfection.

Table 1. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Surface Area (cm^2)	Number of Cells to Seed
T75 Flask	75	3.0 – 6.0 x 10^6
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

Table 2. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Volume (ml)	Plasmid DNA (µg)	Diluent Volume (ml)	PrimeFect™ Reagent (µl)
96-well plate	0.1	0.1	2 x 0.005	0.3
48-well plate	0.3	0.25	2 x 0.015	0.75
12-well plate	0.75	0.75	2 x 0.038	2.25
6-well plate	1.0	1	2 x 0.05	3.0
35 mm dish	1.0	1	2 x 0.05	3.0
60 mm dish	2.8	2.5	2 x 0.10	7.5
10 cm dish	5.0	3 - 4	2 x 0.25	9 -12
T75 flask	8.0	9 -18	2 x 0.40	27 -54
250 ml flask	18	25 - 50	2 x 0.80	75 - 150

Step II. Preparation of PrimeFect™-DNA Complex and Transfection Procedures

For Hela cells, the optimal ratio of PrimeFect™ (µl): DNA (µg) is 3:1. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and PrimeFect™ 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 Hela cells are given in the standard protocol described below.

- For each well, add 0.5 ml of complete medium with serum and antibiotics freshly ~60 minutes before transfection.

- For each well, dilute 0.5 µg of DNA into 25 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to bottom of the tube .

- For each well, dilute 1.5 µl of PrimeFect™ reagent into 25 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

Note: Never use Opti-MEM to dilute PrimeFect™ reagent and DNA, it will disrupt transfection complex.

- Add the diluted PrimeFect™ Reagent immediatelyto the diluted DNA solution all at once.

Note: Do not mix the solutions in the reverse order

- Vortex- mix the solution immediately and spin down briefly to bring drops to bottom of the tube followed by incubation of 15~20 minutes at room tempera-ture to allow PrimeFect-DNA complexes to form.

Note: Never keep the DNA/PrimeFect™ complex longer than 20 minutes

- Add the 50 µl PrimeFect™/ DNA complex drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.

- Remove DNA/PrimeFect™ 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.

Storage: PrimeFect™ Transfection Reagent for Hela Cells is stable for up to 12 months at +4°C. This item shipped at ambient temperature.

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