Plusfector™ Reagent

Plusfector™ Cat. No. AB-PF-0001 Size: 1 ml
Plusfector™ Cat. No. AB-PF-0004 Size: 4 x 1 ml

Store at +4°C (do not freeze)

Description

Lipofector EXT™ Reagents are packaged with Lipofector™ Reagent (Cat. No. AB-LF-Q001) and Plusfector™ Reagent (Cat. No. AB-PF-0001).

Lipofector™ Reagent (Cat. No.AB-LF-Q001) is designed for the transfection of DNA or RNA into eukaryotic cells. Lipofector™ is a polycationic liposomal reagent, offering outstanding transfection efficiency.

Transfection activity can be enhanced by using Plusfector™ Reagent (Cat. No. AB-PF-0001) to pre-complex the DNA or RNA

Guidelines for Transfection

1. Prepare complexes using the amount of DNA or RNA , Plusfector™ and Lipofector™ recommended on below table. Optimizations are necessary. We recommend using serum free medium to dilute Lipofector™, Plusfector™
and DNA or RNA.

To transfect cells in different tissue culture formats, vary the amounts of Lipofector™, Plusfector™, DNA or RNA, cells, and medium as shown in the table 1 and table 2

Table 1. Reagent quantities for general recommendation

Culture vessel	Surface area (cm^2)	Vol. of plating medium	DNA or RNA (µg) in media vol. (µl)	Plusfector™ (µl)) in media vol. (µl)	Lipofector™ (µl) in media vol. (µl)	Medium exchange vol.
96-well	0.3	100 µl	0.1 µg in 25 µl	0.3 µl in 25 µl	0.25 µl in 25 µl	60 µl
48-well	0.7	200 µl	0.2 µg in 50 µl	0.6 µl in 50 µl	0.5 µl in 50 µl	120 µl
24-well	2	500 µl	0.4 µg in 50 µl	1.2 ul in 50 µl	1 µl in 50 µl	300 µl
12-well	4	1 ml	0.8 µg in 50 µl	2.4 ul in 50 µl	2 µl in 50 µl	600 µl
6-well	10	2.5 ml	1.5 µg in 100 µl	4.5 ul in 100 µl	4 µl in 100 µl	1.5 ml
60-mm	20	10 ml	3 µg in 100 µl	9 ul in 100 µl	8 µl in 100 µl	6 ml
100-mm	56	30 ml	6 µg in 200 µl	18 ul in 200 µl	20 µl in 200 µl	20 ml

Table 2. . Reagent quantities for optimizing transfections

Cells	DNA or RNA	Plusfector™	Lipofector™
Censitive cells **Note 1**	0.25 ug	0.5 µl – 1.0 µl	0.5 µl – 1.25 µl
Most cell lines	0.5 ug 0.75 ug	1 µl – 2.5µl 1.5 µl – 3.75 µl	1 µl – 3.0 µl 1.5 µl – 4.5 µl
Suspension and robust cells **Note 2**	1 ug	2 µl – 5 µl	2 µl – 6 µl

Note 1. (Examples are HT1080 and Hela)

Note 2. (Examples are MCF7, Jurkat, HL60 and A549)

2. Don't add antibiotics to media during transfection procedure.

3. 70-90% confluence at the time of transfection is recommended for high efficiency and to minimize cytotoxicity. Optimization should be necessary.

4. Test serum-free media for compatibility with Lipofector™ since some serum-free formulations may inhibit liposomal transfection.

5. To avoid microbial contamination all solutions should be sterile-filtered before use and subsequently be handled under aseptic conditions, as is common practice for handling cell cultures.

Transfection Procedure

Use the following procedure to transfect adherent mammalian cells in a 24- well format. For other formats, see table 1

1. **[Cell culture]** Plate the cells the day before the transfection experiment. The appropriate plating density for a particular cell line will depend on the growth rate and the shape of the cells. The cells should be 70-90% confluent on the day of transfection. As a general guideline, plate 2-6 x 10^4 cells in 500 µl culture medium with the usual amount of serum.

2. **[Medium exchange]** While complexes are forming, remove the growth medium from cells and replace with 250 ul transfection medium without serum (usually the cell growth medium without serum.).

3. **[DNA or RNA dilution]** Dilute 0.4 µg (or 0.25 – 1.0 ug) DNA or RNA in 50 µl of serum free medium (or other appropriate medium) without serum and mix gently.

4. **[Plusfector dilution]** Dilute 1.2 µl (or 0.5 – 5 ul) Plusfector™ in 50 µl of serum free medium (or other appropriate medium) without serum and mix gently.

5. **[Premixing]** Combine the diluted DNA (or RNA) (from step3) and diluted Plusfector™ (from step 4). Mix gently and incubate for 10-15 minutes at room Temperature or 4℃. (approximate total volume = 100 µl)

Note 3. Some plasmid or high density of DNA or RNA are better in 4℃ incubation

6. **[Lipofector dilution]** Dilute 1 µl (or 0.5 – 6 ul) Lipofector™ in 50 µl of AB-OTM™ optimized transfection medium (or other appropriate medium) without serum and mix gently.

7. **[Complexes formation]** Combine the diluted DNA (or RNA) / Plusfector™ (from step 5) and diluted Lipofector™ (from step 6). Mix gently and incubate for 15 - 30 minutes at room Temperature or 4℃. (approximate total volume = 150 µl)

8. **[Transfection]** Add the 150 ul of diluted complexes (DNA or RNA + Plusfector™ + Lipofector™ / from step 7) to each well. Mix gently by rocking the plate.

9. **[Cell culture]** Incubate cells at 37°C in a CO_2 incubator for 4 - 6 hours. Add 400 ul of growth medium containing 2X the normal concentration of serum without removing the transfection mixture. If toxicity is problem, replace medium with fresh, complete medium (with normal amount of serum).