APTABIO™

Lipofector-2000 Reagent

Cat. No. AB-LF-2002 Size: 1.5 ml
Store at +4°C (do not freeze)

Description

Lipofector-2000 Reagent (Cat. No. AB-LF-2002) is designed for the transfection of DNA or RNA into eukaryotic cells. Lipofector-2000 is a polycationic liposomal reagent, offering outstanding transfection efficiency with easy Procedure, in the presence or absence of serum.

Guidelines for Transfection

1. Prepare complexes using the amount of DNA or RNA and Lipofector-2000 recommended on below table. Optimizations are necessary. We recommend using serum free medium to dilute Lipofector-2000 and DNA or RNA.

To transfect cells in different tissue culture formats, vary the amounts of Lipofector-2000, 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)	Lipofector-2000 (μl)
96-well	0.3	100 μl	0.1 μg in 25 μl	0.25 μl
48-well	0.7	200 μl	0.2 μg in 50 μl	0.5 μl
24-well	2	500 μl	0.4 μg in 50 μl	1 μl
12-well	4	1 ml	0.8 μg in 50 μl	2 μl
6-well	10	2.5 ml	1.5 μg in 100 μl	4 μl
60-mm	20	10 ml	3 μg in 100 μl	8 μl
100-mm	56	30 ml	6 μg in 200 μl	20 μl

Table 2.Reagent quantities for optimizing transfections (24 well)

Cells	DNA or RNA	Lipofector-2000
Sensitive cells **Note 1**	0.25 ug	0.5 μl – 1.0 μl
Most cell lines Plasmid expression	0.5 ug 0.75 ug	1 μl – 3.0 μl 1.5 μl – 4.5 μl
Suspension and robust cells **Note 2**	1 ug	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-2000 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. **[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. .

3. **[Lipofector-2000 dilution]** Dilute 1 μl (or 0.5 – 6 ul) Lipofector-2000 in 50 μl of serum free medium (or other appropriate medium) without serum and mix gently.

4. **[Complexes formation]** Combine the diluted DNA (or RNA) (from step 2) and diluted Lipofector-2000 (from step 3). Mix gently and incubate for 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.

5. **[Transfection]** Add the 50 ul of diluted complexes (DNA or RNA + Lipofector-2000 / from step 4) to each well. Mix gently by rocking the plate.

6. **[Cell culture]** Incubate cells at 37°C in a CO_2 incubation for further investigation. If toxicity is problem, replace medium with fresh, complete medium 4 – 6 hours after transfection. (with normal amount of serum).

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