# **APTABIO**<sup>™</sup>

## Lipofector-pMAX Regaent

Cat. No. AB-LF-M100 Size: 1 ml Store at +4°C (do not freeze)

### Description

Lipofector-pMAX Reagent (Cat. No. AB-LF-M100) is designed for the transfection of DNA or RNA into eukaryotic cells. Lipofector-pMAX is a polymer based reagent, offering outstanding transfection efficiency with easy procedure, in the presence of serum.

#### Guidelines for Transfection

**1. [Cell seeding]** 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 60-70 % confluent on the day of transfection. Typically, for transfection in 24-well plate,  $5x10^4$  to  $1x10^5$  cells are seeded per well 24 hours prior to transfection. Change medium 30 minute before performing the experiment and add 400 µl of medium per well. Lipofector-pMAX is stable in the presence of serum therefore you may use serum containing medium during the entire experiment. For other culture formats, refer to Table 1 for recommended number of cells to seed the day before transfection.

Table 1.

Culture plate	Number of cells to seed	Volume of medium per well (ml)	
96-well	1x10 <sup>4</sup> - 2x10 <sup>4</sup>	0.1-0.2	
48-well	2.5x10 <sup>4</sup> - 5x10 <sup>4</sup>	0.2-0.5	
24-well	5x10⁴ - 1x10⁵	0.4-1	
12-well	8x10⁴ - 2x10⁵	1-2	
6-well	2x10⁵ - 4x10⁵	2-4	
6cm/flask 25cm <sup>2</sup>	4x10⁵ - 8x10⁵	5-10	
10cm/flask 75cm <sup>2</sup>	2x10 <sup>6</sup> - 4x10 <sup>6</sup>	10-15	

\*Note: Transfection efficiency may be increased by reducing the volume.

2. [Preparation of the complex and transfection] Prepare complexes using the amount of DNA and Lipofector-pMAX recommended on below Table 2. Optimizations are necessary. To transfect cells in different tissue culture formats, vary the amounts of Lipofector-pMAX, DNA, cells, and medium as shown in the Table 1 and Table 2.

#### Table 2.

Culture plate	Amount of DNA(ug)	Volume of Lipofector-pMAX reagent (ul)	Total volume of complexes added per well (ul)
96-well	0.1	0.2	20
48-well	0.25	0.5	50
24-well	0.5	1	100
12-well	1	2	100
6-well	2	4	200
6cm/flask 25cm <sup>2</sup>	3	6	500
10cm/flask 75cm <sup>2</sup>	5-10	10-20	500

#### **Transfection Procedure**

Use the following procedure to transfect adherent mammalian cells in a 24-well format. For other formats, see Table 1 and 2. We recommend using 2 ul of Lipofector-pMAX per ug DNA as starting condition, however the amount of Lipofector-pMAX may be adjusted from 1 to 4 ul per ug of DNA depending on the cell line to be transfected.

1.[Medium exchange] Change medium 30 minute before performing the experiment and add 400  $\mu l$  of medium per well.

2. **[DNA dilution]** Per well, dilute 0.5 ug of DNA in 150 mM NaCl to a final volume of 50 ul. Voltex gently and spin down briefly.

3. **[Lipofector-pMAX dilution]** Per well, dilute 1 ul of LipofectorpMAX reagent in 150 mM NaCl to a final volume of 50 ul. Voltex gently and spin down briefly.

4. **[Complexes formation]** Add the 50 ul Lipofector-pMAX solution to the 50 ul DNA solution all at once. Please note that mixing the solutions in reverse order may reduce transfection efficiency. Voltex or pipeting the solution immediately and spin down briefly. Incubate for 15 to 30 minutes at room temperature.

5.**[Transfection]** Per well, add the 100 ul Lipofector-pMAX/DNA mix drop-wise to the cells in 400 ul of serum-containing medium and gently swirling the plate.

6. [Cell culture] Return the plate to the cell culture incubator.

7. [**Assay]** Perform reporter gene assay 24 to 48 hours following transfection.