



DNAfectin™ Plus Transfection Reagent

Cat. No. G2500

Store at 4°C.

Product Description

abm's DNAfectin™ Plus Transfection Reagent is a unique formulation of polycations and liposomes, which guarantees **high transfection efficiency** in a wide variety of cell types including primary cells.

Cat. No.	Product	Quantity
G2500	DNAfectin™ Plus Transfection Reagent	1.0ml

Protocol

Use the following conditions as guidelines to transfect mammalian cells in a 6-well or 35mm dish format. For other culture vessels, please refer to Table 1 below.

- 18 to 24 hours prior to transfection, seed cells at a density of 70%. For suspension cells, seed cells at 5×10^5 cells/ml.
- For each transfection sample, prepare the complexes as follows:
Solution A: Dilute 2.0µg of DNA into 100µl of serum-free, antibiotic-free medium.
Solution B: Mix DNAfectin™ Plus by flicking the tube prior use, then dilute 6-10µl of DNAfectin™ Plus in 100µl serum-free, antibiotic-free medium.

Incubate Solution A and Solution B at room temperature for 5 minutes.

- Combine the solutions, mix gently to ensure uniform distribution and incubate for 20 minutes at room temperature.
NOTE: Complexes are stable at room temperature for 3-5 hours.
- Add 0.8ml of serum-free medium to each of DNA-transfection complex, and mixing well by flicking the tube vigorously.
- Aspirate all medium from each well, then add the 1.0ml transfection reagent complex directly to each well (for adherent cells). For suspension cells, collect cells and spin cells at 300xg to collect cell pellet, resuspend with 1.0ml transfection complex. Then transfer cells and transfection complex into a 6-well. Incubate the cells.

- After 5-8 hours, remove transfection solution and add 2.0ml of complete medium (with serum and antibiotics). Continue to incubate the cells.
- 48-72 hours later, transgene expression can be analyzed with an appropriate assay or continue to next step for stable cell line generation.
- To make stable cell lines: subculture cells at densities the same as the density used in killing curve determination in the presence of the selection marker.

Optimizing Transfection for Specific Cell Lines

To achieve the maximum transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density along with DNA and DNAfectin™ concentrations. Optimal results can be achieved when cells were 70% confluent and DNA (µg): DNAfectin™ (µl) ratios were 1:3 to 1:5.

Table 1: Reagent Quantities for Different Culture Vessels

Vessel	Culture volume	DNA	DNAfectin™	Transfection volume
24-well	500µl	0.2-0.4µg in 25µl	0.6-2.0µl in 25µl	0.3ml
12-well	1.0ml	0.5-0.8µg in 50µl	1.5-4.0µl in 50µl	0.4ml
6-well	2.0ml	1.0-2.0µg in 100µl	3.0-10µl in 100µl	1.0ml
35mm	2.0ml	1.0-2.0µg in 100µl	3.0-10µl in 100µl	1.0ml
60mm	5.0ml	3.0-6.0µg in 500µl	9.0-30µl in 500µl	2.0ml
10cm	10ml	8.0-16µg in 1.5ml	24-80µl in 800µl	5.0ml

NOTE: DNAfectin™ Plus performs similar to Life Lipofectamine™ 2000 in terms of gene transduction efficiency and cytotoxicity in most cells. In the event that your specific cells are sensitive to this transfection reagent (>30% cells die within 48 hours after transfection), reducing the transfection reagents and the amount of DNA by 50% will solve the problem while achieving successful gene transduction results.