



Applied Biological Materials Inc

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DNAfectin™ 2100

Store at +4°C

Cat. No.	Description	Quantity
G2100	DNAfectin™ 2100	1.0mg/1.0ml

Description

DNAfectin™ comprises of a unique formulation of polycations and liposomes, which will guarantee high transfection efficiency and low cytotoxicity for any cell type including primary cells. Over 50% transfection efficiency can be achieved using our DNAfectin™ formulation for any cell line.

Transfection 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.

1. Adherent Cells: 18 to 24 hours prior to transfection, seed cells at a density of $1-3 \times 10^5$ cells per well in 2.0ml of appropriate growth medium (with serum and antibiotics if cells are cultured in the presence of them). Incubate the cells at 37°C in a CO₂ incubator until cells are 70% to 90% confluent at the time of transfection.

Suspension Cells: Just prior to preparing complexes, plate $3-5 \times 10^5$ cells in 0.8ml of serum free medium without antibiotics.

Since transfection efficiency is sensitive to culture confluence, it is important to maintain a standard seeding protocol from experiment to experiment.

2. 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: Vortex DNAfectin™ reagent thoroughly prior use, then dilute 10-20 µl of DNAfectin™ reagent in 100µl serum-free, antibiotic-free medium.

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

3. Combine the solutions, mix gently to ensure uniform distribution and incubate for 20 minutes at room temperature. For suspension cells, go directly to step 5.

NOTE: Complexes are stable at room temperature for 3-5 hours.

4. Adherent Cells ONLY: Add 0.8ml of serum-free, antibiotic-free medium to DNAfectin™-DNA complex. Mix solution gently.

5. Adherent Cells: Remove growth medium from the cells and add 1.0ml of DNAfectin™-DNA solution to the each well containing cells.

Suspension Cells: Add 0.2ml of the DNAfectin™-DNA solution into each well containing suspension cells in 0.8ml serum-free, antibiotic-free medium.

6. After 5-8 hours, remove transfection solution and add 2.0ml of the appropriate growth medium (with serum and antibiotics) or add 0.1ml of FBS directly into each vessel.
Incubate the cells at 37°C in a CO₂ incubator for a total of 18-24 hours.
7. To make stable cell lines: Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours post transfection. Selection medium can be added the following day if desired.

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 have been observed when cells were 80-90% confluent and DNA(µg): DNAfectin™ (µl) ratios were 1:1 to 1:5.

Table 1: Reagent Quantities for Different Culture Vessels

Culture Vessel	Surface area per well (cm ²)	Volume of plating medium	DNA(µg) in medium volume (µl)	DNAfectin™ in medium volume	Transfection medium vol.
24-well	2	500 µl	0.2-0.4 µg in 25 µl	2-4 µl in 25 µl	0.4ml
12-well	4	1ml	0.5-0.8 µg in 100 µl	5-8 µl in 100 µl	0.6ml
6-well	10	2ml	1.0-2.0 µg in 100 µl	10-20 µl in 100 µl	0.8ml
35mm	10	2ml	1.0-2.0 µg in 100 µl	10-20µl in 100 µl	0.8ml
60mm	20	5ml	3.0-6.0µg in 500 µl	30-75 µl in 500 µl	2.4ml
10-cm	60	10ml	8.0-16.0 µg in 1.5ml	90-200µl in 800µl	6.4ml

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For technical questions, phone the ABM helpline at 1-866-757-2414
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CERTIFICATE OF ANALYSIS