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# DNAfectin™2100

Store at 4°C

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

**Description**
DNAfectin™2100 comprises of four unique formulations of polycations and liposomes, which will guarantee high transfection efficiency and low cytotoxicity for any cell type including primary cells.

**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 x $10^5$ cells per well in 2.0 ml 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 $CO_2$ incubator until cells are 70% to 90% confluent at the time of transfection.

   *Suspension Cells:* Just prior to preparing complexes, plate 3-5 x $10^5$ cells in 0.8 ml 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 2.0 µg of DNA into 100 µl of serum-free, antibiotic-free medium.

   *Solution B:* Vortex DNAfectin™2100 thoroughly prior use, then dilute 10-20 µl of DNAfectin™2100 in 100 µl 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™ 2100-DNA complex. Mix solution gently.

5. *Adherent Cells:* Remove growth medium from the cells and add 1.0ml of DNAfectin™ 2100-DNA solution to the each well containing cells.

*Suspension Cells:* Add 0.2 ml of the DNAfectin™ 2100-DNA solution into each well containing suspension cells in 0.8 ml serum-free, antibiotic-free medium.

6. After 5-8 hours, remove transfection solution and add 2.0 ml of the appropriate growth medium (with serum and antibiotics) or add 0.1 ml of FBS directly into each vessel. Incubate the cells at 37˚C in a $CO_2$ 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 the 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™2100 (µl) ratios were 1:1 to 1:5.

**Table 1: Reagent Quantities for Different Culture Vessels**

| Culture Vessel | Volume of plating medium per well | DNA(µg) in medium volume (µl) | DNAfectin™2100 in medium volume (µl) | Transfection medium vol. |
|---|---|---|---|---|
| 24-well | 500 µl | 0.2-0.4 µg in 25 µl | 2-4 µl in 25 µl | 0.4 ml |
| 12-well | 1 ml | 0.5-0.8 µg in 100 µl | 5-8 µl in 100 µl | 0.6 ml |
| 6-well | 2 ml | 1.0-2.0 µg in 100 µl | 10-20 µl in 100 µl | 0.8 ml |
| 35mm | 2 ml | 1.0-2.0 µg in 100 µl | 10-20 µl in 100 µl | 0.8 ml |
| 60mm | 5 ml | 3.0-6.0µg in 500 µl | 30-75 µl in 500 µl | 2.4 ml |
| 10-cm | 10 ml | 8.0-16.0 µg in 1.5ml | 90-200 µl in 800µl | 6.4 ml |