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## **PRODUCT INFORMATION**

### **DNAfectamine**

#### ***Product information for DNAF001:***

##### **Description**

DNAfectamine Transfection Reagent 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.

##### **Application**

Transfection of DNA into cultured eukaryotic cells.

##### **Kit Contents**

1ml at 1.0mg/1.0ml

##### **Storage**

Store at 4°C. Do not freeze.

##### **Note**

Please check our Transfection Cell Database for tested cell lines.

##### **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 CO<sub>2</sub> incubator until cells are 70% to 90% confluent at the time of transfection.
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 DNAfectamine reagent thoroughly prior use, then dilute 10-20 µl of DNAfectamine 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.  
*NOTE: Complexes are stable at room temperature for 3-5 hours.*
4. Add 0.2ml of the DNAfectamine solution from step 4 into each well containing cells in 0.8ml

serum-free, antibiotic-free medium.

5. After 12-16 hours (overnight), change to complete medium.

6. 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 Transfection Reagent concentrations. Optimal results have been observed when cells were 80-90% confluent and DNA( $\mu$ g): Transfection Reagent ( $\mu$ l) ratios were 1:1 to 1:5.

Table 1: Reagent Quantities for Different Culture Vessels

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

### Transfection Database

Our Transfection Cell Database contains data from transduction results from cell lines tested. This is a continuing effort and more cell lines will be added to the database once the data is available. Please search our database below for specific details.

| Cell Line | Cell Line Species/Tissue | Nucleic Acid | Reagent                      | Transfection Type | Transfection Efficiency(%) |
|-----------|--------------------------|--------------|------------------------------|-------------------|----------------------------|
| 293T      | Human Embryonic Kidney   | DNA          | DNAF001 Transfection Reagent | Transient         | 100%                       |
| Jurkat    | Human T Lymphoma         | DNA          | DNAF001 Transfection Reagent | Transient         | 25%                        |
| SHEP      | Human Neuroblastoma      | DNA          | DNAF001 Transfection Reagent | Transient         | 90%                        |
| BOSC23    | Human Kidney             | DNA          | DNAF001 Transfection Reagent | Transient         | 90%                        |
| HT1080    | Human Fibrosarcoma       | DNA          | DNAF001 Transfection Reagent | Transient         | 80%                        |
| C33A      | Human Cervical Carcinoma | DNA          | DNAF001 Transfection Reagent | Transient         | 90%                        |
| MRC5      | Human Primary Fibroblast | DNA          | DNAF001 Transfection Reagent | Transient         | 80%                        |

|               |                                    |     |                              |           |         |
|---------------|------------------------------------|-----|------------------------------|-----------|---------|
| MDK-Telo      | Dog Kidney Cells+Telo              | DNA | DNAF001 Transfection Reagent | Transient | 80%     |
| B16           | Murine Melanoma                    | DNA | DNAF001 Transfection Reagent | Transient | 80%     |
| CB3           | Mouse Erythroleukemia              | DNA | DNAF001 Transfection Reagent | Transient | 25%     |
| HuVEC         | Human Ubilical Vein                | DNA | DNAF001 Transfection Reagent | Transient | 20%     |
| HepG2         | Human Liver Carcinoma              | DNA | DNAF001 Transfection Reagent | Transient | 40%     |
| MMRU          | Human Melanoma                     | DNA | DNAF001 Transfection Reagent | Transient | 75%     |
| MMAN          | Human Melanoma                     | DNA | DNAF001 Transfection Reagent | Transient | 80%     |
| MDA-MB-231    | Human Breast Carcinoma             | DNA | DNAF001 Transfection Reagent | Transient | 85%     |
| HeLa          | Human Cervical Carcinoma           | DNA | DNAF001 Transfection Reagent | Transient | 80%-90% |
| A2780         | Human Ovarian Carcinoma            | DNA | DNAF001 Transfection Reagent | Transient | 70%-75% |
| L6            | Rat Myoblast                       | DNA | DNAF001 Transfection Reagent | Transient | 60%-70% |
| Rabbit-telo   | Telomerase-Rabbit Cells            | DNA | DNAF001 Transfection Reagent | Transient | 50%     |
| USO2 (T-USO2) | Human Osteosarcoma (tet-inducible) | DNA | DNAF001 Transfection Reagent | Transient | 80%-90% |

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