Instructions for use

# DOTAP

**Reagent for the liposome-mediated transfection of eucaryotic cells**

## 1. General description

The transfection reagent DOTAP is an aqueous solution of liposomes formed by the lipid N-[1-(2,3-**D**i**o**leoyloxy)]-N,N,N-**t**rimethyl**a**mmonium **p**ropane methylsulfate.
Its concentration is 1 mg/ml.

| | |
|---|---|
| Molecular weight: | 774,21 |
| Molecular formula: | $C_{43}H_{83}NO_8S$ |
| Purity, QC: | Control via TLC, NMR, min 99 %<br>Standard transfection assay with CV-1-cells. |
| Shelf life: | 6 months, when stored at 4 °C. |
| Culture media: | Transfects nucleic acids both in the presence and absence of serum. |
| Storage | 4 °C |
| Shipping: | At room temperature |

Only for research purposes in vitro, not intended for human or animal diagnostic, therapeutic, or other clinical uses.

**Important information:**
Do not freeze, mix gently before use.

## 2. Instructions for use

### *2.1 Purity of nucleic acid*

It is essential for a high transfection efficiency to use only very pure DNA or RNA ($OD_{260/280}$ = 1,5-1,8). We recommend a purification by column chromatography or by cesium chloride gradient centrifugation. After purification the nucleic acid should be free both of traces of cesium and endotoxins.

### *2.2 Optimization of transfection efficiencies*

Successful transfection requires careful optimization of transfection conditions for each cell type. Cells used for transfection experiments should not be contaminated (free of mycoplasmae) and proliferate well, before being plated at a constant density. This is necessary to obtain reproducible transfection rates. We recommend using regularly passaged cells to ensure a permanent growth phase. Also it is recommended to subcultivate the cells the day before performing the transfection experiment. Transfection efficiencies can be increased by optimization of the following parameters: Presence or absence of serum, concentrations of the DOTAP reagent and the nucleic acid (DNA or RNA), the ratio DOTAP/nucleic acid and the length of the transfection period. All these parameters are considered in our optimization protocol in paragraph 4.

## 3. Transfection protocols

### *3.1 Required reagents*

- DOTAP transfection reagent
- sterile stock solution of nucleic acid (e.g. 10 mM Tris-HCl, 1 mM EDTA, pH 7.4)
- sterile culture medium, serum-free and free of antibiotics
- optionally: 20 mM HEPES (cell culture grade), pH 7.4, sterile
- sterile culture medium containing serum (free of antibiotics)
- sterile culture medium containing serum and antibiotics

### *3.2 Transfection of adherent cells*

**I) Preparation of adherent cells**

*(refering to a 6-well plate, respectively 35 mm culture dish)*
Plate per well 1.0 - 3.0 x $10^5$ cells suspended in 1-2 ml culture medium (containing serum and antibiotics). Cultivate the cells at 37 °C in a $CO_2$-incubator until confluency has reached 60-80 %. This commonly takes 18-24 h, however the incubation period may vary according to the different cell lines.

**II) Preparing the DOTAP / nucleic acid-complex**

Information: Please don´t use HEPES buffer. HEPES will diminish your transfection rate.

Dilute 2.5 µg nucleic acid in medium free of serum and antibiotics to a final concentration of 0.1 µg/µl (final volume 25-50 µl). Please use a sterile vessel (e.g. 96 well microtest plate or tubes made of polystyrene or glass). Pipette 16 µl DOTAP transfection reagent in a separate sterile well or tube and adjust the volume to 50 µl with medium free of serum and antibiotics.
Transfer the nucleic acid solution to the DOTAP/serum-free medium solution and **mix gently** by pipetting the mixture several times.

**Please note: Do not vortex or centrifuge!**

Incubate the transfection mixture (DOTAP/nucleic acid-complex) for 10 to max. 15 min at room temperature.

**III) Transfection with serum**

Preparing the transfection medium:
Mix carefully the transfection mixture with 1-2 ml culture medium containing serum (free of antibiotics).
Transfection of the cells:
Remove culture medium. Wash the cells in medium containing serum (free of antibiotics). Add transfection medium.
Thereafter incubate the cells for 3-6 h under their specific growth conditions.
Replace the transfection medium by fresh culture medium containing serum and antibiotics. The following period of incubation (commonly 24-72 h) depends on the gene of interest (e.g. reporter genes) respectively the promotor that you use. Examples of vectors containing reporter genes are pCMV ß-Gal, pCMV SPORT ß-Gal, pSV2-CAT, pCMV-CAT etc.).

**IV) Serum-free transfection**

Preparing the transfection medium:
Mix carefully the transfection mixture with 1-2 ml culture medium free of serum and antibiotics.
Transfection of the cells:
Remove culture medium and wash the cells carefully 3 times in medium free of serum and antibiotics.
Add the transfection medium to the cells and continue as in step III.

If the cultivating conditions are changed for the transfection procedure (e.g. use of serum-free medium instead of medium containing serum), it is often beneficial to cultivate the cells prior to transfection for at least 1-5 h under the modified conditions.

### *3.3 Transfection of cells in suspension*

**I) Preparation of cells in suspension**

Passage the cells before the day of transfection. 1.0 - 3.0 x $10^5$ cells/ml and a final volume of approx. 5 ml of fresh culture medium gave best results.

**II) Preparing the DOTAP / nucleic acid-complex**

Information: Please don´t use HEPES buffer. HEPES will diminish your transfection rate.

Dilute 5 µg nucleic acid in medium free of serum and antibiotics to a final concentration of 0.1 µg/µl (final volume 50-100 µl). Use a sterile tube (e.g. 96 well microtest plate or tube made of polystyrene or glass). Pipette 32 µl DOTAP transfection reagent in a separate sterile well or tube and adjust the volume to 100 µl with medium free of serum and antibiotics.

Transfer the nucleic acid solution to the DOTAP / serum-free medium solution and **mix gently** by pipetting the mixture several times.

**Attention: Do not vortex or centrifuge!**

Incubate the transfection mixture (DOTAP / nucleic acid-complex) for 10 to max. 15 min at room temperature.

**III) Transfection with serum**

Preparing the transfection medium:
Mix carefully the transfection mixture with 2-5 ml serum containing culture medium (free of antibiotics).
Transfection of the cells:
Wash the cells carefully medium with serum (free of antibiotics). Centrifuge for 10 min at 250 x g.

After the washing step resuspend the cells in transfection medium. Transfer the cell suspension to a culture vessel (e.g. 75 ml culture flask or 60 mm culture dish) and incubate the cells for 3-6 h under their specific growth conditions.
Replace the transfection medium by fresh culture medium. The following period of incubation (commonly 24-72 h) depends on the gene of interest (e.g. reporter genes) respectively the promotor that you use. Examples of vectors containing reporter genes are pCMV ß-Gal, pCMV SPORT ß-Gal, pSV2-CAT, pCMV-CAT etc.).

**IV) Serum-free transfection**

Preparing the transfection medium:
Mix carefully the transfection mixture with 2-5 ml culture medium free of serum and antibiotics).
Transfection of the cells:
Wash the cells carefully 3 times in serum-free medium (without antibiotics). Therefore, centrifuge for 10 min at 250 x g. Remove the supernatant between the steps and add fresh medium (free of serum and antibiotics). After the last washing step resuspend the cells in transfection medium and continue as in step III.

## 4. Optimization of transfection rates

Transfection efficiencies may vary between different cell lines.
The following parameters can be optimized:

Presence or absence of serum:
In presence of serum the formation of the DOTAP/nucleic acid-complex is inhibited. Nevertheless, it is stable in the presence of serum. Therefore, the transfection can be carried out in the presence or absence of serum. The result depends on the specific cell line.

DOTAP/nucleic acid complex formation in cultrure medium or HEPES buffer
Please don´t use HEPES buffer. HEPES will diminish your transfection rate.

Incubation time:
Commonly 3-10 h of incubation are sufficient to obtain high transfection rates. The period of incubation can be prolonged up to 72 h, because the DOTAP/nucleic acid-complex doesn´t show any cytotoxic effects within this range. Working with prolonged transfection time requires lower cell density at the beginning of transfection (e.g. lower than 60-80% confluency for adherent cells). The appropriate plating density depends on the period of time between transfection and reporter gene analysis (longer period = lower density, shorter period = higher density).

Ratio DOTAP/nucleic acid:
The amount of DOTAP can be optimized within the range of 5-20 µl per µg nucleic acid.

Concentrations of DOTAP and nucleic acid:
The maximum DOTAP concentration should not exceed 40 µl/ml culture medium.
The amount of nucleic acid can be optimized within a range of 0,5-2,5 µg refering to a 35 mm culture dish.

## 5. Overview of transfection protocols

| | Adherent cells | | | Cells in suspension |
|---|---|---|---|---|
| Diameter of culture dish | 35 mm | 60 mm | 100 mm | |
| Number of cells | 60-80 % confluency on the day of transfection | | | |
| Volume per culture dish | 1-2 ml | 5-6 ml | 10-14 ml | 2-5 ml |
| Amount of nucleic acid | approx. 2.5 µg | approx. 5 µg | Approx. 7.5 µg | approx. 5 µg |
| Final volume of nucleic acid solution | 25-50 µl | 50-100 µl | 75-150 µl | 50-100 µl |
| Volume of DOTAP reagent | 16 µl | 32 µl | 48 µl | 32 µl |
| Final volume of DOTAP / serum-free medium solution | 50 µl | 100 µl | 140 µl | 100 µl |

| | | |
|---|---|---|
| **DOTAP** | 0.5 ml | **L787.1** |
| | 1.0 ml | **L787.2** |
| | 5.0 ml | **L787.3** |

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