# DOTAP

## The established Transfection Reagent for Mammalian Cells

For ordering information, SDS, publications and application notes see www.biontex.com

| Product | Order No. | Size |
|---|---|---|
| DOTAP | T010–1.0 | 1.0 ml |
| DOTAP | T010–2.0 | 2 x 1.0 ml |
| DOTAP | T010–5.0 | 5 x 1.0 ml |

**Shipping:** At room temperature

**Storage:** 4°C (**do not freeze**)

**Stability:** Best before: see label

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

### Description

DOTAP is a monocationic transfection reagent based on liposome technology. These liposomes are formed by the cationic lipid N-[1-(2,3-Dioleoyloxy)]-N,N,N-trimethylammonium propane methylsulfate (C43H83NO8S, MW: 774,21). Its concentration is 1 mg/ml in a ready-to-use aqueous solution. DOTAP transfects nucleic acids in the presence and absence of serum.

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# 1. General Guidelines

## 1.1 Specifications

| | |
|---|---|
| **Application** | Transfection of mammalian cells with DNA or antisense oligos |
| **Formulation** | 1 mg/ml 1,2-Di-(9Z-octadecenoyl)-3-trimethylammoniumpropane methyl sulfate in water |
| **Assays** | Up to 150 (6-well) or up to 75 (60 mm) per 1 ml reagent |
| **Sterility** | tested |
| **Cell Culture** | tested |
| **Storage** | 4°C |

## 1.2 Quality Control

Standard transfection assay. Absence of bacterial and fungal contamination is verified using thioglycolate medium.

## 1.3 Explanatory Remarks

### Storage

DOTAP is **delivered non-chilled and should be stored in a cooler at approx. 4°C immediately after receipt**.

Storage for a short period at room temperature is not a problem provided that the reagent is subsequently stored again at 4°C. Do not freeze!

### State of cells

Cells to be transfected should be well proliferating and healthy. Cells which have been in a quiescent state at confluency for a while (before seeding) may not be transfected as efficiently as cells which are growing rapidly. Therefore it is recommended to use regularly passaged cells for transfection experiments. Microbial contamination, for example with mycoplasma or fungi, can drastically alter transfection results.

***Antibiotics*** must be avoided where indicated. In some cases cell death can be caused by use of antibiotics in the transfection medium.

### Cell confluency

The true confluency of the cells (adherent) to be transfected cannot be estimated visually by using a microscope, but can be optimally determined by means of a growing curve and comparison with counted cells. In many cases a 90-100% covered growing area is correlated with 30-60% true confluency.

> The DNA transfection during the exponential growing phase of the cells is essential for optimum results, because of the critical role of cell division in transport of the DNA into the nucleus. The optimal confluency has to be adapted to the cell line used!

### Quality of nucleic acids

To achieve optimum transfection results the DNA or RNA used should be of the maximum possible purity. Bacterial related contamination such as endotoxins will significantly impair transfection efficiency.

### Adsorption processes

Before forming a complex with DOTAP the DNA and the reagent should not be kept in solution in serum-free medium for longer than 5 minutes. Adsorption of the DNA and the lipid by the vessel material may impair transfection efficiency. Polypropylene shows a minimum tendency towards adsorption of transfection reagent and genetic material in comparison to e.g. glass and polyethylene. For the same reason, the lipoplex should be added to the cells immediately after the specified incubation period. Adsorption processes also impair downscaling and upscaling processes because of the differing ratios of plastic surface area to volume of medium in vessels of varying sizes.

### Optimization

Although DOTAP shows a broad peak performance, if optimal results are desired we recommend optimization of the transfection protocol for each combination of plasmid and cell line used. Every cell line has its characteristically optimal DNA/lipid ratio; even the format of dishes can influence the ratio or absolute amounts of reagents. Additionally, a protocol used for other transfection reagents should never be transferred to DOTAP (or any other different transfection reagent). Every transfection reagent possesses its own molecular structure, with specific physical properties which have an important influence on DNA/lipid ratios.

### Stable transfection

If you desire Stable Transfection, follow the usual working instructions for seeding cells with lower density. On the day of transfection, cells should preferably be less than 50% confluent. After the transfection procedure, replace transfection medium with a suitable selected medium containing antibiotics.

# 2. Working Instructions

**ⓘ For first use, we recommend an optimization protocol.**

## 2.1 Transfection of Adherent Cells

1. In a 35-mm tissue culture plate, seed 2.5-10.0 x $10^5$ (starting point: 5.0 x $10^5$) cells per dish in 1-2 ml of the appropriate complete growth medium.
2. Incubate the cells at 37°C in a $CO_2$ incubator until the growing area is 90-100 % covered. The time required will vary among cell types, but will usually take 18-24 hours.
3. The solutions of DNA or RNA and DOTAP transfection reagent should have ambient temperature and be gently vortexed prior to use.
4. Prepare the following solutions using for example a 96-well plate (cell culture grade). **Medium must be pipetted first.** Pure solutions must not come into contact with plastic surfaces:
   - Solution **A:** 1-3 µg of DNA to 100 µl medium (free of serum and antibiotics), starting point: 2 µg
   - Solution **B:** 2-30 µl of DOTAP transfection reagent to 100 µl medium (free of serum and antibiotics), starting point: 16 µl
5. Mix the solutions gently by carefully pipetting several times.
6. Add solution A to solution B, mix gently by carefully pipetting several times (do not vortex or centrifuge!), and incubate at room temperature for 15-20 min. This time is required to form the DNA-lipid complex.
7. While complexes form rinse the cells once with 1-2 ml PBS or appropriate medium and refill the plate with 1.0 ml fresh antibiotics-free medium with serum (or without, if serum free conditions are desired).

8. Add the DNA-lipid complexes to the cells and mix gently. Incubate the cells with the complex for 3-10 hours at 37°C in a $CO_2$ incubator (starting point: 6 hours).
9. Following incubation, replace the transfection medium by 1.5 ml complete growth medium.
10. Depending on cell type and promoter activity, assay cells for gene activity 24-72 hours following the start of transfection.

## 2.2 Transfection of Suspension Cells

1. Wash the cells once with PBS or appropriate medium.
2. In a 35 mm tissue culture plate, seed 1.0-6.0 x $10^6$ cells (starting point: 2.0 x $10^6$) in 2 ml fresh antibiotic-free medium with serum, or without serum if serum-free conditions are desired.
3. Prepare the following solutions using for example a 96-well plate (cell culture grade). **Medium must be pipetted first.** The solutions of DNA or RNA and DOTAP transfection reagent should have ambient temperature and should be gently vortexed prior to use.

   Solution **A**: 5 µg of DNA in 100 µl medium (free of serum and antibiotics), starting point: 2 µg

   Solution **B:** 20-50 µl of DOTAP transfection reagent in 100 µl medium (free of serum and antibiotics), starting point: 32 µl
4. Mix the solutions gently by carefully pipetting several times.
5. Combine the two solutions (pipet solution A into solution B), mix gently by carefully pipetting several times (do not vortex or centrifuge!), and incubate at room temperature for 15-20 min. This time is required to form the DNA-lipid complex.
6. Add the DNA-lipid complexes to the cell suspension and mix gently.
7. Incubate 3-10 hours at 37°C in a $CO_2$ incubator (starting point: 6 hours).
8. Replace the medium with fresh complete medium.
9. Depending on cell type and promoter activity, collect cells by centrifugation and assay cells for gene activity 24-72 hours following the start of transfection.

# 3. Optimization

## 3.1 Critical optimization parameters

### Ratio of DNA to DOTAP

The most important optimization parameter is the ratio of DNA or RNA to DOTAP.

For successful transfection a slightly net positive charge of the DNA/DOTAP complex is required. The optimal DNA/DOTAP ratio depends on the cell line. The amount of DOTAP can be optimized within the range of 5-20 µl per µg 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 for a 35 mm culture dish.

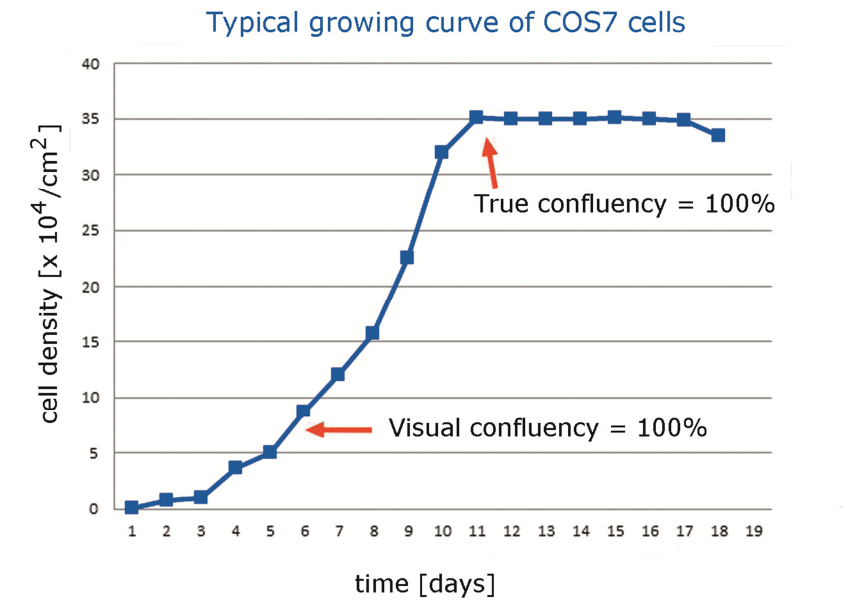
### Quantity of transfection complex

In order to obtain the highest transfection results, optimization of the concentration of DNA-lipid-complex may be required. An excessive amount of the complex can lead to overexpression and/or lysis of cells (lipids are also lysis reagents!).

Optimal ratio of DNA/DOTAP and concentration of DNA-lipid complex may vary with the number of cells. To ensure reproducible optimization of these parameters, it is necessary to maintain a constant number of seeded cells and incubation period until the addition of the lipoplex.

## Cell confluency

Good results are regularly obtained with a 90-100% covered growing area. In general, transfecting procedure should be matched to the exponential growth phase of the cells because of the important role of cell division in transporting the DNA into the nucleus. Nevertheless, the optimal confluency depends on the cell line used.




As a rule, confluency determined visually ("visual" confluency = percentage of growth surface covered with cells) is not identical with confluency determined by growing curve (= true confluency). The best results are obtained when transfection is performed at the highest possible proliferation state (= 30-60% true confluency). This often corresponds to visual confluency of 90-100%.

## Effect of serum

A further important parameter is the effect of serum. Most of the cell lines transfected with DOTAP showed superior results when transfection was performed in the **presence** of serum.

Nevertheless, some cell lines show different behaviour. Accordingly, transfection can be performed without serum, under serum-reduced (e.g. 5%) or full-serum (e.g. 10%) conditions.

Presence of serum during **complex formation** between DOTAP and DNA is **strictly forbidden** as serum may inhibit complex formation. Once the complex is formed, contact with serum is permitted.

Optimization of these critical parameters should give satisfactory results.

## 3.2 Further optimization parameters

These parameters can further be optimized by a step-by-step procedure.

### Incubation time with transfection complex

Cells can be exposed to the transfection complex within a variety of time ranges from 3-72 hours. Usually a transfection time range of between 3-10 hours is sufficient. The optimum exposure time to the transfection mixture is dependent on the sensitivity of the transfected cell line. With very sensitive and/or highly proliferating cells, the addition of fresh complete culture medium or replacement in such medium after transfection has favourable effects.

### Time range between transfection and evaluation

Assay for gene activity should be performed 24-72 hours after the start of transfection. The optimal time is dependent on cell type, promoter activity and expression product (e.g. toxicity).

# 4. Up- and Downscale

*Table: Reagent quantities for different sizes of culture vessels (proposed starting points for a standard protocol in brackets).*

| | **Adherent cells (subculture the day before transfection)** | | | **Cells in suspension** |
|---|---|---|---|---|
| **Diameter of culture dish** | 35 mm | 60 mm | 100 mm | |
| **Number of cells per dish* [x10$^6$]** | 30-60% true confluency on the day of transfection | | | |
| | 0.25–1.0 (0.5) | 0.6–2.4 (1.2) | 1.5–6.0 (2.5) | 0.5 – 3.0 x 10$^5$/ml |
| **Volume of culture medium per dish** | 2 ml | 5 ml | 12 ml | 2-5 ml |
| **Amount of nucleic acid** | approx. 2.5µg | approx. 5µg | approx. 7.5µg | approx. 5 µg |
| **Final volume of nucleic acid solution** | 100 µl | 200 µl | 300 µl | 100 µl |
| **Starting volume of DOTAP reagent** | 16 µl | 32 µl | 48 µl | 32 µl |
| **Final volume of DOTAP / serum-free medium solution** | 100 µl | 200 µl | 300 µl | 100 µl |

* Numbers of cells to seed depend on cell type and size. Optimization may be necessary. Maintain same seeding conditions between experiments.

# 5. Miscellaneous

## 5.1 Important Information

This reagent is developed and sold for research purposes and *in vitro* use only. It is not intended for human or animal therapeutic or diagnostic purposes.

## 5.2 Warranty

Biontex guarantees the performance of this product until the date of expiry printed on the label when stored and used in accordance with the information given in this manual. If you are not satisfied with the performance of the product please contact Biontex Laboratories GmbH.

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