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DOTAP

Catalog Number	Size
DT10-1	1.0 ml
DT10-2	2 x 1.0 ml
DT10-5	5 x 1.0 ml

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. It is intended for the transfection of mammalian cells with DNA or antisense oligos.

Application: Transfection of mammalian cells with DNA or antisense oligos.

Formulation: 1 mg/ml 1,2 – Di(9Z-octadecenoyl)-3-trmethylammoniumpropane methyl sulfate in water

Assays: Up to 150 (6-well) or up to 75 (60 mm) per 1 ml reagent

Shipping: Shipped at room temperature.

Storage: +4°C **(do not freeze)**

Stability: See label for expiration date.

Note: This product is for research use only. Not for use in human or animal diagnostics, therapeutics, or clinical applications.

General Guidelines

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 with mycoplasma or fungi can drastically alter transfection results.

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.

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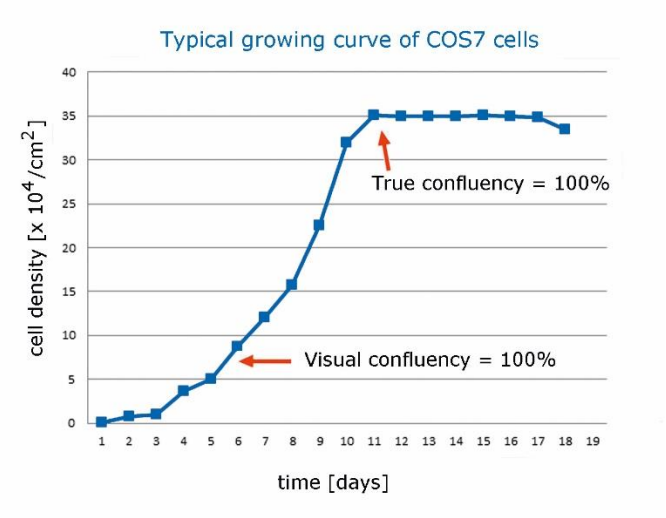
Confluency determined visually ("visual" confluency = % of growth surface covered with cells) is **not identical** with confluency determined by a growing curve (true confluency). Best results are obtained if transfection is performed at the highest possible proliferation state (= 30-60% true confluency). This often corresponds with visual confluency of 90-100%.



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 the Nucleic Acid

DNA or RNA should be of the highest purity if optimal results for transfection are desired. For example, endotoxins decrease transfection efficiency. Bacterial related contamination such as endotoxins will significantly impair transfection efficiency.

Antibiotics

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

Adsorption Process

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 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 rations of plastic surface area to volume of medium in vessels of varying sizes.

Optimization

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DOTAP shows a broad peak performance, but if optimal results are desired it is recommended to optimize the transfection protocol for each combination of plasmid and cell line used. Every cell line has a characteristically optimal DNA or RNA-lipid ratio. The format of dishes for lipoplex formation and cell culture can influence the ration and absolute amounts of reagents (likely caused by differing adsorption properties of the tube material based on differing surface dimensions).

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 or RNA-lipid ratios. Appropriate optimization instructions are given in this manual. As a rule, only little optimization is required if the recommended starting points are used.

Stable Transfection

If stable transfection is desired, 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.

Transfection Protocol

Optimization - determination of a growth curve may be necessary, please see Optimization Protocol.

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 DNA or RNA stock solutions and DOTAP transfection reagent should be at room temperature and gently vortexed before use.
4. Prepare the following solutions using a cell culture grade 96-well plate or other tubes made of polypropylene, glass, or polystyrene (preferably polypropylene). **Medium must be pipetted first.** Pure solutions must not come into contact with plastic surfaces.
   Solution A: 1 – 3 µg of DNA or RNA to 100 µl serum and antibiotic-free medium, starting point: 2 µg.
   Solution B: 2 – 30 µl of DOTAP transfection reagent to 100 µl serum and antibiotic-free medium, starting point: 16 µl.
5. Mix each solution gently by carefully pipetting one time.
6. Add **Solution A to Solution B** and mix carefully by pipetting several times (do not vortex or centrifuge). Incubate at room temperature for 15 – 20 minutes 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 antibiotic-free medium with serum (serum free conditions can also be used).
8. Add the DNA-lipid complexes to the cells and mix gently. Incubate the cells with complex for 3 – 10 hours at 37°C in a CO_2 incubator (starting point: 6 hours).

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9. Following incubation, replace the transfection medium by 1.5 ml complete growth medium.
10. Depending on the cell type and promoter activity, assay cells for gene activity 24 – 72 hours following the start of transfection.

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. The DNA or RNA stock solutions and DOTAP transfection reagent should be at room temperature and gently vortexed before use.
4. Prepare the following solutions using a cell culture grade 96-well plate or tubes made of polypropylene, glass or polystyrene (preferably polypropylene) for each transfection. **Medium must be pipetted first.** Pure solutions must not come into contact with plastic surfaces.
   Solution A: 5 µg of DNA or RNA to 100 µl serum and antibiotic-free medium, starting point: 2 µg.
   Solution B: 20 – 50 µl of DOTAP transfection reagent to 100 µl serum and antibiotic-free medium, starting point: 32 µl.
5. Mix each solution gently by carefully pipetting several times.
6. Add **Solution A to Solution B** and mix carefully (do not vortex or centrifuge). Incubate at room temperature for 15 – 20 minutes to form the DNA-lipid complex.
7. Add the DNA-lipid complexes to the cell suspension and mix gently.
8. Incubate 3 – 10 hours at 37°C in a CO_2 incubator (starting point: 6 hours)
9. Replace the medium with fresh complete medium.
10. Depending on the cell type and promoter activity, assay cells for gene activity 24 – 72 hours following the start of transfection.

Optimization

Critical Optimization Parameters

Ratio of DNA or RNA 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 or RNA-DOTAP complex is required. The optimal DNA or RNA-DOTAP ratio depends on the cell line. The amount of DOTAP can be optimized within 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.

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Quantity of transfection complex

In order to obtain the highest transfection results, optimization of the absolute amount of DNA or RNA- lipid complex may be required.

Optimal ratio of DNA or RNA to DOTAP and concentration of DNA or RNA-lipid complex may vary with the number of cells. An excessive amount of the complex can lead to over expression and/or lysis of cells (lipids are also lysis reagents). It is necessary to keep the number of seeded cells and incubation period constant until the transfection procedure for a reproducible optimization of these parameters.

Cell Confluency

See General Guidelines section.

Effects of serum

To date, nearly all cell lines transfected with DOTAP have shown superior results if transfection is performed in the presence of serum. Special cell lines may show different behavior. Transfection can be performed without serum, under serum reduced (e.g 5%), or full serum (e.g. 10%) conditions.

There must be no serum present during **complex formation** between DOTAP and DNA or RNA. Serum may inhibit complex formation. Once the complex is formed, contact with serum is permitted.

Optimal ratio of DNA or RNA to DOTAP and concentration of DNA or RNA-lipid complex may vary with different serum concentrations. Optimization of these critical parameters by following the optimization protocol should give satisfactory results.

Additional Optimization Parameters

These parameters can be further 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 (i.e. 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 favorable 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).

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Up and Downscale

Reagent quantities for different sizes of culture vessels (proposed starting points for optimization in parenthesis) are indicated.

	ADHERENT CELLS (SUBCULTURE THE DAY BEFORE TRANSFECTION)			CELLS IN SUSPENSION
DIAMETER OF CULTURE DISH	35 mm	60 mm	100 mm	
	30-60% TRUE CONFLUENCY ON THE DAY OF TRANSFECTION			
NUMBER OF CELLS PER DISH* [X10^6]	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.