Transfection

Frequently Asked Questions

In reference to transfection of genetic material (DNA) with synthetic cationic lipids.

"What are the factors that determine whether a cell is easy, difficult or impossible to transfect?"

"I have always used a different transfection reagent. Can I now use Metafectene with the same protocol as my old reagent?"

"Can the transfectability of a specific cell type be predicted?"

"What are the ideal cell culture conditions for delivering optimum transfection results?"

"How can I determine the optimum parameters for lipoplex formation?"

"I have noted toxic effects in my transfection processes. What is the cause?"

"How does the serum used in the culture medium influence transfection success?"

"Do mycoplasmas have an effect on transfection success?"

"How can I find out whether my cells are contaminated with mycoplasma?"

"What effect do the type of DNA/RNA and its quality have on transfection success?"

"Does the material of my culture or reaction vessels play a significant role?"

"I plan to change to a different cell culture format. What should I keep in mind?"

"Does Biontex offer specific protocols for specific cell lines and transfection reagents?"

"Biontex transfection reagents are shipped unrefrigerated. Does this affect their properties?"

Cambio Ltd, 1 The Irwin Centre, Scotland Road, Dry Drayton, Cambridge, CB23 8AR, UK
Tel :+44 (0)1954 210 200 , +44 (0)1954 210 300, email: support@ cambio.co.uk
www.cambio.co.uk

"Although Biontex transfection reagents are shipped unrefrigerated, the product labeling and manuals give different specifications for storage temperatures. Why is this?"

"Are Biontex transfection reagents autofluorescent?"

"What are the factors that determine whether a cell is easy, difficult or impossible to transfect?"

A critical role is played by the ability of a specific cell type to undergo endocytosis and proliferate, since the lipoplexes initially escape into the cytosol via endocytosis and are there released from the endosomes. Lipoplexes / decomplexed DNA can only penetrate to the cell nucleus (i.e. overcome the "nuclear barrier") if the nuclear membrane is permeable for a short time during division. If one of the two processes occurs in a poorly developed form, transfection efficiency may be lowered accordingly even if application of the transfection method is optimal. It is thus unsurprising that cells with low proliferation - such as primary or nerve cells - or cells with low endocytosis activity - such as J774 cells - are regarded as difficult to transfect. A further key factor is the intracellular cytosolic nuclease activity which is specific to each individual type of cell. If this is extremely high, it may cause the decomplexed DNA in the cytosol to degrade completely within a very short space of time. Proliferation and nuclease activity are two processes critical to transfection efficiency which may show extremely different characteristics depending on the cell type. An exacerbating factor is that the factors given above may differ completely not only from cell type to cell type, but also among different genotypes of the same cell type. Differing genotypes may appear in cells of the same name in different universities or even different working groups within a single institution, and can also be observed during long-duration passaging of a cell line. This complexity reduces the options for transferring protocol parameters from one user to another to the minimum, even if apparently the same cell type is involved.

"I have always used a different transfection reagent. Can I now use Metafectene with the same protocol as my old reagent?"

No; every transfection reagent has a set of specific properties, including:

1. Liposome / micelle forming substances
2. Aggregate stability / lipoplex stability (melting point)
3. Lipoplex structure
4. Specific compositions and proportions of colipids
5. Specific net load per molecule and pH

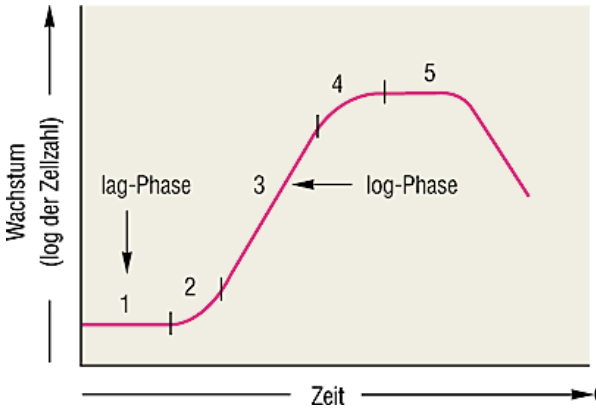
These differing properties have a direct and specific influence on their interaction with the DNA and the cell type in question. Because of this, protocol parameters cannot be transferred from one reagent to another.

"Can the transfectability of a specific cell type be predicted?"

No, this is not possible. The interactions of the highly specific properties of every cell type and every transfection reagent are so complex that predictions of transfection efficiency are futile. Only a statistical evaluation of case studies can provide a certain level of probability for the tendency to deliver better or worse results. For the transfection reagents in common use today, a relatively comprehensive optimization study must be conducted in order to identify the potential transfectability of the user cell type with a specific reagent. The only reagent on the market which can be used without optimization is the third-generation transfection reagent METAFECTENE EASY, a completely new development by Biontex. This reagent enables transfectability to be determined extremely rapidly for any type of cell.

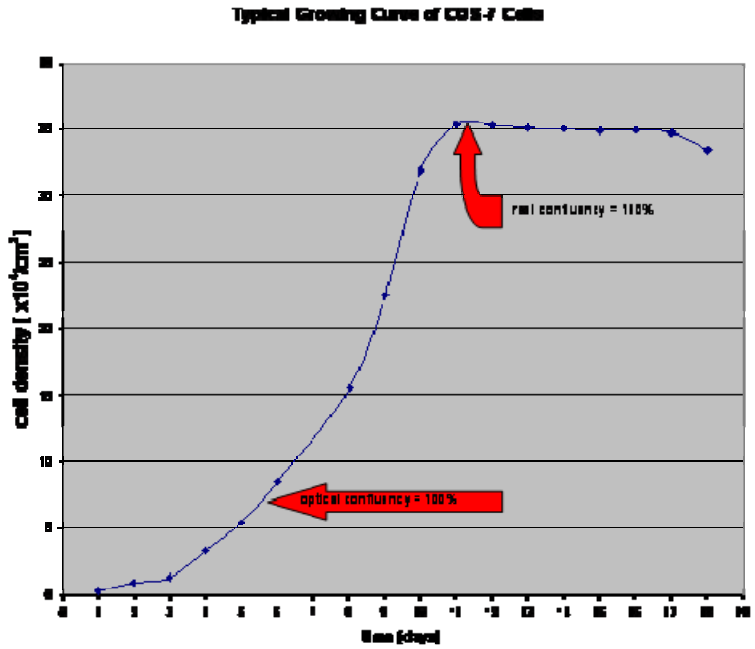
"What are the ideal cell culture conditions for delivering optimum transfection results?"

- A basic principle in transfection of DNA with synthetic carrier systems is that this material can only penetrate the "nuclear barrier" during cell division. As an unavoidable result, the following aspects must be observed:
- The number of cell culture passages should not exceed 30. A certain genetic differentiation takes place with each passage; this means that cells best able to resist the stress of passaging will proliferate disproportionately. In addition, the composition of the cell culture medium changes the physiological status of the cells, since the cells align themselves to the existing nutrient conditions. This causes the genetic divergences from the original cells to widen progressively.
- At the time of transfection cells should grow as fast as possible. Cells should be permanently maintained in a state of high proliferation, known as the log or exponential phase. This phase can be achieved most easily by creating a growing curve for the cell type to be used and for the appropriate format (a 96-cell format or below cannot generally be used because of the specific surface conditions), and thus setting the optimum cell amount and growth duration. Owing to growth inhibition, cells that are periodically maintained in a confluent condition only regain their full growth potential after several passages.



Growth (log of cell numbers)
Lag phase / Log phase / Time

- Optical confluence is not the same as actual confluence. The growing curve above indicates the extent to which visually determined confluence (where the whole growth surface is covered) corresponds to actual confluence (Phase 5 of the growing curve). These two confluence values generally diverge significantly. Taking the example of COS7 cells shown in the diagram, it is clear that in the most frequent case only the early growth phase 3 is achieved. For this reason, cells should have a visually determined confluence of around 90% on the day before transfection if the process is to be performed on adherent cells.



"How can I determine the optimum parameters for lipoplex formation?"

Cambio Ltd, 1 The Irwin Centre, Scotland Road, Dry Drayton, Cambridge, CB23 8AR, UK
Tel :+44 (0)1954 210 200 , +44 (0)1954 210 300, email: support@ cambio.co.uk
www.cambio.co.uk

Lipoplex morphology is closely linked to the type of cationic lipid and the lipid composition of the transfection reagent used on the one hand, and on the ratio of genetic material to transfection reagent on the other. Each cell type shows specific levels of compatibility with the transfection reagent used and the resulting lipoplex morphologies. Although this compatibility is not predictable, the following parameters for optimum transfection can be determined by applying an optimization process:

- First the optimum ratio of DNA/RNA to lipids must be determined. It is generally assumed that lipoplexes require a positive net charge to be capable of electrostatic interaction with the negatively charged cell membrane surface for successful endocytotic absorption. In most cases this ratio approximates 1-7µl of lipid to 1µg DNA/RNA.
- The second key factor is the amount of lipoplex per cell; if this is too high the resulting toxicity over-compensates for the success of transfection. The amount of lipoplex should thus be varied to reflect the known optimum ratio of DNA/RNA to lipid.

"I have noted toxic effects in my transfection processes. What is the cause?"

The properties of lipids are similar to those of cell membranes; this is probably the main reason for increased toxicity as the amount of lipids in the lipoplexes increases. For this reason, high levels of lipids in relation to the amount of DNA/RNA can only be used to a limited extent. For reasons as yet unknown, lipoplexes have a significantly greater toxic impact on cells than do the transfection reagents themselves. This results in the second limitation: excessive lipoplex levels can impair transfection success. A further reason for toxicity is the desired cell manipulation itself, which may have major effects on cell physiology. In this case, optimum transfection may lead to high cell mortality, which can only be avoided by downregulating transfection efficiency. The third significant factor is the gene or gene product itself to be expressed or inhibited via siRNA. If this cell manipulation causes major physiological disruption to the cell, a well-functioning transfection process may actually generate toxic effects. In these cases, the amount of lipoplex used should be reduced (maintaining the same RNA/DNA to lipid ratio).

"How does the serum used in the culture medium influence transfection success?"

The serum used in the cell culture (e.g. FCS, FBS) is a highly complex undefined mixture of water-soluble blood components such as growth factors and nutrients, and always has a significant influence on transfection success.

- Serum has extensive lipoplex-inhibiting properties. Because of this, serum must not be present during lipoplex formation (after the transfection reagent has been added to the genetic material).
- With modern reagents, serum does not affect lipoplexes already formed. Second-generation transfection reagents in particular (METAFECTENE, METAFECTENE PRO, METAFECTENE EASY, METAFECTENE SI) have overcome the problem of lipoplex inhibition by serum; the lipoplexes formed with these transfection reagents can be added to the cells together with serum-containing cell culture medium without impairing transfection results. In fact, these reagents regularly deliver enhanced levels of transfection efficiency when serum is present.
- Serum supports cell growth behavior. Its components, such as growth hormones, significantly increase growth and thus the cell division rate of the cells to be transfected, which in turn significantly improves transfection efficiency.
- Because sera have an extremely limited shelf-life (maximum 1 month), care should be taken that the sera used are within their expiry date, since the use of expired sera would significantly impair the ingredients needed by the cells. In this case cells show a completely different and reduced growth pattern (see diagram) and optimum transfection results can no longer be anticipated.
- Sera may be contaminated with mycoplasmas and nucleases. Nucleases destroy any form of genetic material within a very short time, while mycoplasmas manipulate cells in a way which impacts extremely negatively on transfection success. While many manufacturers supply sera free from nuclease activity, practically all specify that the mycoplasma levels in their products are below detection sensitivity thresholds. This is completely insufficient for cell culture applications, as a single mycoplasma cell is enough to contaminate a cell culture. Biontex supplies a range of MycoFREE sera which are both nuclease-free and, thanks to an especially developed process, guaranteed mycoplasma-free, making contamination impossible.
- Sera are unknown compounds and may vary from batch to batch. This variability of serum components may result in extensive variations in transfection results, even if all other known influence parameters can be stabilized. To avoid this influence parameter, Biontex can reserve large volumes of FBS from a single batch.

"Do mycoplasmas have an effect on transfection success?"

Yes, since any contamination of the cell culture, whether with fungi, viruses or bacteria, has a major impact on transfection results, generally causing the entire cell culture to die. Since mycoplasma contamination has the special characteristic that it is invisible and does not cause cell death, contamination may go undetected for very long periods. Nevertheless, these bacteria have an extensive impact on transfection success:

- Cell growth is significantly reduced owing to increased requirements of arginine (see diagram)
- Mycoplasmas modulate cell cytokine production
- Mycoplasmas cause chromosomal aberrations
- Mycoplasmas lodge in the cell membranes and may modulate cell membrane processes such as endocytosis.

Mycoplasmas can proliferate in almost all known cell types and are thus ubiquitous. Roughly 80% of all cell cultures from Japan, 65% from Argentina and 15% from the USA are estimated to be contaminated with mycoplasmas. The main sources of contamination are trypsin and FBS, both cell culture additives produced from animal sources. The majority of manufacturers which supply these media guarantee only mycoplasma levels below the detection threshold, which is a completely inadequate criterion. A single mycoplasma cell is enough to contaminate a whole cell culture. Biontex bietet hierfür über ein spezielles Verfahren zu 100% dekontaminiertes Trypsin MycoVIR und FBS MycoFREE an. Damit ist jegliche Kontamination der Zellkultur durch Mykoplasmen ausgeschlossen. Biontex supplies 100% decontaminated Trypsin MycoVIR and FBS MycoFREE, produced using a special process, which enable mycoplasma contamination to be ruled out completely.

"How can I find out whether my cells are contaminated with mycoplasma?"

A long-standing detection method for mycoplasma is DAPI staining. However, this method only shows up significant levels of contamination owing to its extremely low detection sensitivity (see under Mycoplasma-free cell culture). The PCR method is significantly more sensitive and is thus the detection method of choice. Biontex offers the MycoSPY full detection kit which enables even single copies of the mycoplasma genome to be identified. If contamination is detected, the bacteria can be eliminated simply and cheaply by using the MycoRAZOR.

"What effect do the type of DNA/RNA and its quality have on transfection success?"

Basically genetic material with a higher level of purity will always deliver better transfection results. A factor to watch for in particular is contamination by lipopolysaccharides known as endotoxins. They are introduced during the manufacturing process by bacteria and, even when present in minute amounts, can trigger toxic effects in mammalian cells, thus significantly impairing the transfection process. Care should be taken that cleaning of the genetic material includes the removal of endotoxins, for which commercial kits are available. Plasmid cleaning based on "miniprep protocols" is not recommended for transfection purposes, as the specific structure of the genetic material directly impacts on transfection success:

- Because promotors have specific expression rates, cells have a characteristic amount, or amount per unit of time, of expression product.
- Specific characteristics of the gene to be expressed or of the resulting protein may, depending on cell type, significantly impact on the physiology of the cell or even cause cell mortality.

In addition, different proteins are not broken down at equal rates by proteases in cell cytosol, so that half-life may vary depending on the protein and cell type. This means that the transfection success for each gene and its attendant protein is a matter of chance, particularly given the cell-specific interplay of highly complex interactions described above. The expression time at which cell protein levels reach their maximum must thus be redetermined for each cell and protein individually in combination with all other factors. The dimensions and tertiary structure of the genetic material also influence potential transfection success. In general, the following principles apply:

- A cell's transfectability declines in line with the amount of genetic material
- Supercoiled plasmids are more efficient in transient transfection, while linear DNA is more suitable for stable transfection.

"Does the material of my culture or reaction vessels play a significant role?"

Yes, since transfection reagents, genetic material and their aggregates – lipoplexes – are surfactants and can adsorb to the vessel surface. Results of our tests show the following suitability chart:

Teflon> Polypropylene>Polystyrol>Polycarbonate>Glass

As Teflon is extremely expensive and thus an uncommon choice for cell culture applications, polypropylene should be the vessel material of choice. All molecules involved are at minimum composed of highly charged molecular components or components with additional hydrophobic properties (known as amphiphilicity). The type of interaction which is critical for transfection success is adsorption, which irreversibly removes the transfection material from the process. An additional complicating factor is that each kind of surface material adsorbs the above substances to a different extent, so that the ratio of reagent to genetic material may differ widely at different times during the process of lipoplex formation, resulting in lipoplexes with differing compositions and in differing amounts. Standardizable transfection results must therefore be based on an essential standardization of cell culture vessel material (both material and manufacturer), vessel formats, material used for pipet tips, and incubation periods both for individual substances in the vessel in question and for the lipoplexes themselves until their addition to the cells.

"I plan to change to a different cell culture format. What should I keep in mind?"

- Optimum parameters determined for a specific vessel format cannot be simply applied in proportion to other vessel formats.
- The smaller the vessel format used, the higher the lipoplex amount / mm^2 must be. When downscaling from a 6-well dish to a 96-well format, approximately three times the amount of lipoplex / cm^2 must be used to achieve comparable transfection efficiency.
- The smaller the vessel format used, the higher lipid/DNA ratio needed.

Specific surface materials and their influences on the transfection process are detailed above ("Culture vessel material") These surface characteristics play a special role in upscaling and downscaling. In upscaling, the vessel wall surface increases as a square factor, while the volume of solution to be used is cubed. This ensures that the surface characteristics exert an increasingly minor influence on the individual agents, and thus on the transfection process as a whole. The reverse applies in downscaling.

"Does Biontex offer specific protocols for specific cell lines and transfection reagents?"

No. Given the large number of parameters given in the FAQs as influential factors in successful transfection, it is clear that supplying specific protocols for general use would be futile. The published case studies listed on our website can only give indications of how to achieve successful transfection as rapidly as possible, and generally cannot claim to be universally applicable.

"Biontex transfection reagents are shipped unrefrigerated. Does this affect their properties?"

Not at all. All previous experiences, including many long-term tests, have shown that storage of the reagents at temperatures between 25 and 40 °C for a period of two weeks did not result in impaired properties of the reagents or reduction of shelf life to below the specified expiry date. Refrigerated shipping would be extremely disadvantageous: given that each application requires the adaption of the reagent to room temperature, the properties of the reagents would show significant negative change.

"Although Biontex transfection reagents are shipped unrefrigerated, the product labeling and manuals give different specifications for storage temperatures. Why is this?"

The delivery time does not affect the product's shelf life within the expiry date. However, longer periods at higher temperatures than those specified may

accelerate or continue ageing processes. For this reason, the products should be stored at the optimum specified storage temperature as soon as possible after delivery (and also after every use), to retain product quality.

"Are Biontex transfection reagents autofluorescent?"

No; the molecular structure of the reagent ingredients renders them incapable of fluorescence. However, we have received occasional reports of autofluorescence, a phenomenon also observed with other transfection agents. On the basis of today's scientific knowledge there is no explanation for this occurrence.

Cambio Ltd, 1 The Irwin Centre, Scotland Road, Dry Drayton, Cambridge, CB23 8AR, UK
Tel :+44 (0)1954 210 200 , +44 (0)1954 210 300, email: support@ cambio.co.uk
www.cambio.co.uk