K4® Transfection System

DNA & RNA Transfection Kit for Mammalian Cells

For order information, SDS, publications and application examples see www.biontex.com

Product	Order No.	Size
K4® Transfection System	T080-0.2	K4® Transfection Reagent 1x0.2 ml K4® Multiplier 1x0.5 ml
K4® Transfection System	T080-1.0	K4® Transfection Reagent 1x1.0 ml K4® Multiplier 1x3.5 ml
K4® Transfection System	T080-2.0	K4® Transfection Reagent 2x1.0 ml K4® Multiplier 2x3.5 ml
K4® Transfection System	T080-5.0	K4® Transfection Reagent 5x1.0 ml K4® Multiplier 5x3.5 ml

Shipping: Room temperature

Storage: K4® Reagent 4°C
K4® Multiplier 4°C (***do not freeze***)

Stability: Best before: see label.
Formulations of liposomes like the K4® Transfection Reagent change their size distribution after long storage at 4°C, which can have slightly adverse effects on the transfection efficiency. This effect can be reversed by a freeze-thaw cycle. We recommend performing a freeze-thaw cycle with the reagent (not the K4® Multiplier) before first use and subsequently monthly to yield optimal results.

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

Description

Eukaryotic cells can detect foreign substances such as lipopolysaccharides, foreign DNA or RNA and foreign proteins and take defensive action against invasion by potential pathogens. Moreover, they use transmitter substances to warn neighboring cells of an attack by potentially cell-damaging substances. Transfection is always governed by these cell-specific defense mechanisms, which frequently significantly impair transfection success.

The K4® Transfection System consists of the K4® Transfection Reagent, which is based on powerful cationic lipids, and the K4® Multiplier, which decreases the cells' ability to detect foreign nucleic acids and can increase transfection efficiency and viability as a result.

The K4® Transfection System is a further development of the K2® Transfection System.

Index

1. General Information

1.1 Specifications

Application	Mammalian cell transfection with DNA and RNA
Assays	300 - 1400 (48-well) with 1.0 ml K4® Transfection Reagent
Sterility*	tested
Cell Culture **	tested
Storage	4°C

* Thioglycolate test
** Standard transfection test

1.2 Storage

K4® Transfection System is supplied **uncooled and all individual constituents should be stored in a refrigerator at 4°C after receipt**. Storage for several days at room temperature is not a problem provided that the constituents are subsequently stored again at 4°C. Freeze-thaw cycles do not affect the constituents. On the contrary, a freeze thaw cycle can reoptimize the gradually changing size distribution of the liposomes in K4® Transfection Reagent.

1.3 Cell conditions

Well proliferating and healthy cells are the easiest to transfect. Regularly passaged cells are therefore a major precondition for success in transfection experiments. Microbial contamination, especially mycoplasma, can drastically impair transfection results.

1.4 Cell confluency

Every cell line has its specific optimal range of amounts for nucleic acids, optimal DNA – lipid ratio or optimal RNA - lipid ratio related to the number of cells used.

For transfection of DNA, the proliferation stage of the cells must additionally be considered. Best results are usually obtained where the coverage of the cell growth area is at 90–100%. At this stage of growth cell proliferation is at its strongest and allows uptake of the DNA into the nucleus, a prerequisite of expression, through the breakup and rebuilding of the core membrane during cell division.

Confluency of adherent cells should not be determined by visual inspection of the growth area under a microscope, but ideally by creating a growth curve for every cell type under the corresponding culture conditions and comparing it to the cell count.

Optimum DNA transfection results can only be achieved by timing the experiment for the exponential growth phase of the cells. The cell division rate plays a key role for the transport rate of the DNA into the cell nuclei. The growth phase is of minor importance for the transfection of RNA.

1.5 Quality of nucleic acids

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

1.6 Adsorption processes

Before forming a complex with K4® Transfection Reagent the DNA or RNA 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. 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.

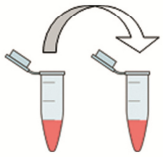
2. Working Instructions

2.1 Work plan

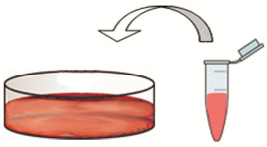
1.
Seeding and growing of the cells (24h)

2.
Add K4®Multiplier to the cells, incubate for 1/2 h

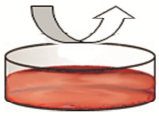
3.
Dilute the nucleic acid (Solution A) and the K4®Transfection Reagent (Solution B) in **serum-free medium**



4.
Preparation of the lipoplex: Add solution A to solution B, incubate for 15 - 20min



5.
Dispense the lipoplex onto the cells, incubate for 6-24h



6.
Replace the transfection medium with fresh medium

7.
Analyze results after 12 - 72h

2.2 Transfection of cells with DNA - standard protocol for 48-well-format -

1. Plate 0.75 – 1.25 x 10^5 adherent cells (starting-point 1.0 x 10^5) or 2.0 x 10^5 suspension cells in a single well of a 48-well dish in 0.25 ml of suitable complete growth medium.*
2. Incubate the cells for 24h at 37°C in a CO_2 incubator. For adherent cells the surface should then be covered 90–100%.

> ⓘ **For first use, we recommend the optimization protocol given in section 2.3.**

Working with a lower cell density requires the reduction of the amount of DNA to avoid toxic effects.

3. Bring the stock solutions of K4® Transfection System and DNA to room temperature and agitate gently to ensure they are evenly mixed.
4. 1/2 h before adding the lipoplex, pipet 2.5 µl K4® Multiplier (1% related to complete growth medium) into each well of cells to be transfected.
5. Prepare the following solutions in a polypropylene vessel. **Always place the medium in the vessel first** to prevent the reagent and DNA solutions from coming into direct contact with the vessel material.

   Solution **A:** 0.3 µg DNA to 15 µl **serum-free** medium

   Solution **B:** 1.2 µl K4® Transfection Reagent to 15 µl **serum-free** medium
6. Mix each solution by gently pipetting up and down once.

For successful transfection of classical suspension cells without extra-cellular matrix, like haematopoietic cells or derived cell lines a much higher amount of DNA and K4® Transfection Reagent can be necessary.

7. Combine the solutions, mix by gently pipetting up and down once, and incubate the mixture at room temperature for 15–20 min.
8. Immediately after incubation add the DNA-lipid complex to the cells, mix gently by agitating the cell culture vessel and incubate in a CO_2 incubator at 37°C.
9. Optional: Remove the transfection mixture after 6–24 h and replace with fresh complete growth medium.
10. Test for reporter gene activity, depending on cell type and promotor activity, between 24–48 h after addition of the lipoplex.

* Cell seeding counts depend on the cell type and must be optimized - ideally by creating a growth curve (see 3.1 Key optimization parameters – Cell seeding counts).

2.3 Optimization protocol of DNA transfection for 48-well-format

Use a suitable reporter gene plasmid, e.g. pCMV-ßGal, pCMV-Luc, pEGFP etc. Ideally, a growth curve to determine the optimum cell seeding count is available. The cells must be at their highest proliferation rate at the point of lipoplex addition.

1. Plate 0.75 – 1.25 x 10^5 adherent cells (starting-point 1.0 x 10^5) or 2.0 x 10^5 suspension cells as a starting point in each well of a 48-well dish in 0.25 ml of suitable complete growth medium.*
2. Incubate the cells for 24 h in a CO_2-incubator at 37°C. For adherent cells the surface should then be covered 90–100%.

Working with a lower cell density requires the reduction of the amount of DNA to avoid toxic effects.

3. Bring the K4® Transfection Reagent, the K4® Multiplier and the DNA solution to room temperature and agitate gently to produce homogenous solutions.
4. Add 2.5 µl of the K4® Multiplier (1% related to complete growth medium) to the cells and incubate for 1/2 h.
5. Prepare the following solutions in a polypropylene vessel. **Always place the medium in the vessel first** to prevent the reagent and DNA solutions from coming into direct contact with the vessel material:

Solution **A**:	add 20 µg DNA to 1000µl **serum-free** medium
Solution **B(1:2)**:	add 9 µl K4® Transfection Reagent to 225 µl **serum-free** medium
Solution **B(1:3)**:	add 13.5 µl K4® Transfection Reagent to 225 µl **serum-free** medium
Solution **B(1:4)**:	add 18 µl K4® Transfection Reagent to 225 µl **serum-free** medium
Solution **B(1:5)**:	add 22.5 µl K4® Transfection Reagent to 225 µl **serum-free** medium

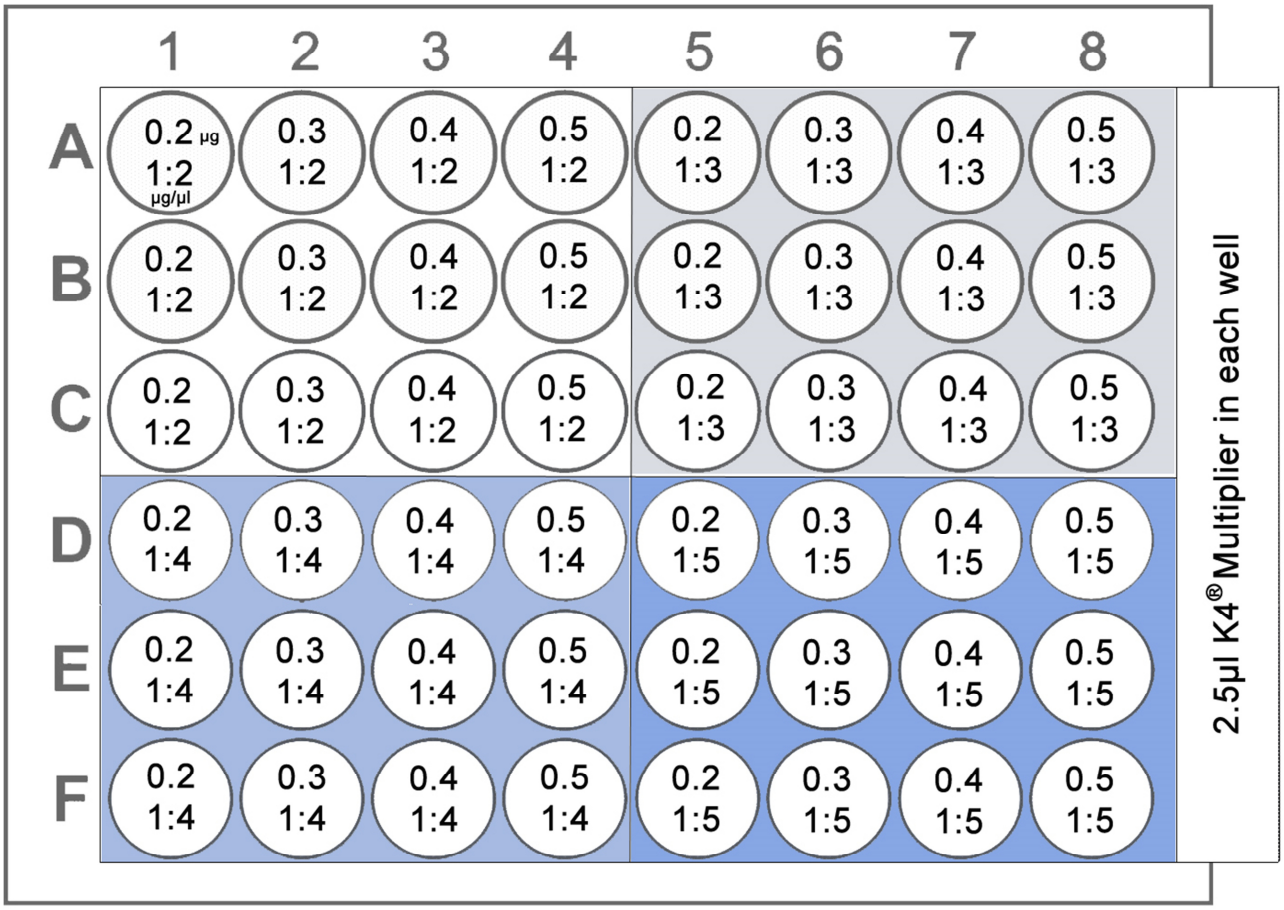
6. Mix each solution by gently pipetting up and down once.
7. Add 225 µl of **A** to the **B** solutions by pipetting, mix by gently pipetting up and down once, and incubate the mixtures at room temperature for 15–20 min.
8. Add the DNA-lipid complexes to the cells immediately after incubation:

20 µl, 30 µl, 40 µl and 50 µl of **A B(1:2)** to A1-A4, B1-B4 und C1-C4
20 µl, 30 µl, 40 µl and 50 µl of **A B(1:3)** to A5-A8, B5-B8 und C5-C8
20 µl, 30 µl, 40 µl and 50 µl of **A B(1:4)** to D1-D4, E1-E4 und F1-F4
20 µl, 30 µl, 40 µl and 50 µl of **A B(1:5)** to D5-D8, E5-E8 und F5-F8

Mix gently by agitating the cell culture vessel and incubate in a CO_2 incubator at 37°C.

* Cell seeding counts depend on the cell type and must be optimized - ideally by creating a growth curve (see 3.1 Key optimization parameters – Cell seeding counts).

The 48-well plate now contains the following combinations:



DNA amount in [µg]
DNA/K4® Transfection Reagent –ratio in [µg/µl]

9. Optional: Remove the transfection mixture after 6–24 h and replace with fresh complete growth medium.
10. Test for reporter gene activity, depending on cell type and promotor activity between 24–48 h after addition of the lipoplex.

2.4 Transfection of cells with mRNA - standard protocol for 48-well-format -

1. Plate 0.5 x 10^5 adherent cells or 1.5 x 10^5 suspension cells in a single well of a 48-well dish in 0.25 ml of suitable complete growth medium.
2. Incubate the cells for 24 h at 37°C in a CO_2 incubator.
3. Bring the stock solutions of K4® Transfection System and mRNA to room temperature and agitate gently to ensure they are evenly mixed.

For first use, we recommend the optimization protocol given in section 2.5.

4. 1/2 h before adding the lipoplex, pipet 2.5 µl K4® Multiplier (1% related to complete growth medium) into each well of cells to be transfected.
5. Prepare the following solutions in polypropylene vessels. **Always place the medium in the vessel first** to prevent the reagent and mRNA solutions from coming into direct contact with the vessel material.

   Solution **A:** 0.4 µg mRNA to 15 µl **serum-free** medium
   Solution **B:** 0.8 µl K4® Transfection Reagent to 15 µl **serum-free** medium

6. Mix each solution by gently pipetting up and down once.
7. Combine the solutions, mix by gently pipetting up and down once, and incubate the mixture at room temperature for 15–20 min.
8. Immediately after incubation add the mRNA-lipid-complex to the cells, mix gently by agitating the cell culture vessel and incubate in a CO_2 incubator at 37°C.
9. Optional: Remove the transfection mixture after 6–24 h and replace with fresh complete growth medium.
10. Test for reporter gene activity between 12–72 h after addition of the lipoplex.

2.5 Optimization protocol of mRNA transfection for 48-well-format

Use a suitable mRNA, coding for a reporter gene like ßGal, Luc, GFP etc.

1. Plate 0.5 x 10^5 adherent cells in each well of a 48-well dish in 0.25 ml of suitable complete growth medium. For suspension cells start with 1.5 x 10^5 cells.
2. Incubate the cells for 24 h in a CO_2-incubator at 37°C.
3. Bring the K4® Transfection Reagent, the K4® Multiplier and the mRNA solution to room temperature and agitate gently to produce homogenous solutions.
4. 1/2 h before adding the lipoplex, pipet 2.5 µl K4® Multiplier (1% related to complete growth medium) into each well of cells to be transfected.
5. Prepare the following solutions in polypropylene vessels. **Always place the medium in the vessel first** to prevent the reagent and mRNA solutions from coming into direct contact with the vessel material:

   Solution **A**: 30 µg mRNA to 700µl **serum-free** medium
   Solution **B(1:2)**: 30 µl K4® Transfection Reagent to 330 µl **serum-free** medium
   Solution **B(1:3)**: 44 µl K4® Transfection Reagent to 330 µl **serum-free** medium

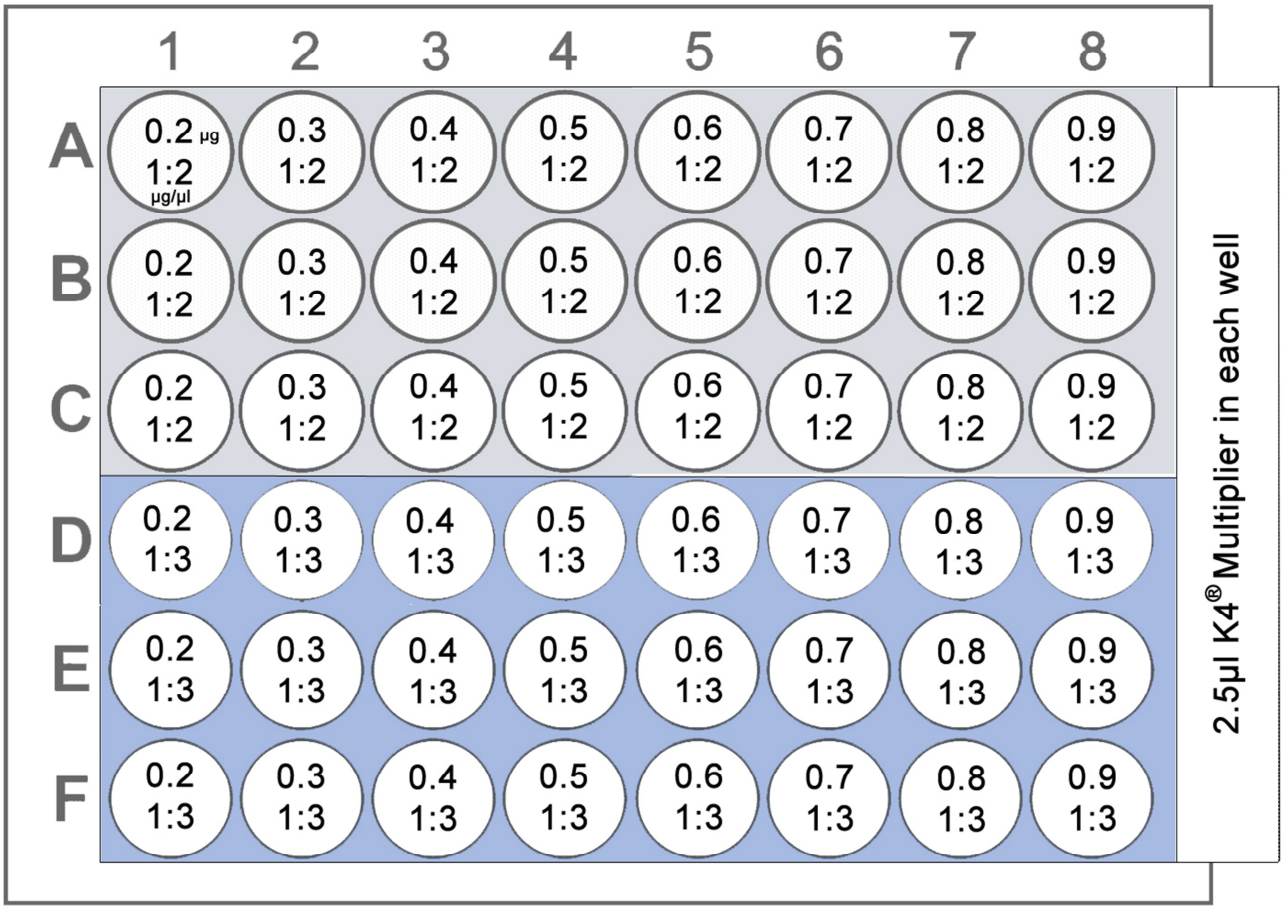
6. Mix the solutions by gently pipetting up and down once.
7. Add 350 µl of **A** to the **B** solutions by pipetting, mix by gently pipetting up and down once, and incubate the mixture at room temperature for 15–20 min.

8. Add the mRNA-lipid-complexes to the cells immediately after incubation:

   10 µl, 15 µl, 20µl, 25µl, 30µl, 35µl, 40 µl and 45 µl of **A B(1:2)** to A1-A8, B1-B8, C1-C8
   10 µl, 15 µl, 20µl, 25µl, 30µl, 35µl, 40 µl and 45 µl of **A B(1:3)** to D1-D8, E1-E8, F1-F8

   Mix gently by agitating the cell culture vessel and incubate in a CO_2 incubator at 37°C.

The 48-well plate now contains the following combinations:



mRNA amount in [µg]
mRNA/ K4® Transfection Reagent –ratio in [µg/µl]

9. Optional: Remove the transfection mixture after 6–24 h and replace with fresh complete growth medium.
10. Test for reporter gene activity between 12–72 h after addition of the lipoplex.

2.6 Transfection of cells with mi/siRNA - standard protocol for 48-well-format -

1. Plate 0.5 x 10^5 adherent cells or 1.5 x 10^5 suspension cells in a single well of a 48-well dish in 0.25 ml of suitable complete growth medium.
2. Incubate the cells for 24 h at 37°C in a CO_2 incubator.
3. Bring the stock solutions of K4® Transfection System and RNA to room temperature and agitate gently to ensure they are evenly mixed.
4. 1/2 h before adding the lipoplex, pipet 2.5 µl K4® Multiplier (1% related to complete growth medium) into each well of cells to be transfected.
5. Prepare the following solutions in polypropylene vessels. **Always place the medium in the vessel first** to prevent the reagent and RNA solutions from coming into direct contact with the vessel material:

   Solution **A:** 0.4 µg mi/siRNA (= 30 pmol) to 15 µl **serum-free** medium
   Solution **B:** 0.8 µl K4® Transfection Reagent to 15 µl **serum-free** medium

6. Mix each solution by gently pipetting up and down once.
7. Combine the solutions, mix by gently pipetting up and down once, and incubate the mixture at room temperature for 15–20 min.
8. Immediately after incubation add the RNA-lipid-complex to the cells, mix gently by agitating the cell culture vessel and incubate in a CO_2 incubator at 37°C.
9. Optional: Remove the transfection mixture after 6–24 h and replace with fresh complete growth medium.
10. Test for reporter gene activity between 12–72 h after addition of the lipoplex.

For optimizing purposes use the half or double amount of lipoplexes and/or use a RNA-reagent-ratio of 0.4µg : 1.2 µl instead of 0.4µg : 0.8 µl.

2.7 Up- and downscaling

Up- and downscaling to various formats can be carried out on the basis of standard parameters or (preferably) optimized parameters and the general proportional areas.

- The cell count seeded per well is determined by the optimal cell count/cm^2.*
- Only the nucleic acid amount must be adjusted specifically, owing to the differing adsorption rates at the vessel walls and the different ratios of surface to volume. Use the multiplying factors shown in each case.
- The nucleic acid-lipid ratio is for all vessels the optimal value.
- The values in brackets correspond to the proposed starting points.

DNA-Transfection

Proposed starting points in brackets

Culture vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate	60mm plate
Growth area [cm^2]	0.31	1.0	1.9	3.7	9.0	22.0
Cell count of adherent cells seeded [× 10^5]	0.2-0.4 (0.3)	0.75-1.25 (1.0)	1.5-2.5 (1.9)	3-5 (3.7)	7-11 (9.0)	15-30 (22.0)
Cell count of suspension cells seeded [× 10^5]	0.3-1.3 (0.9)	1.0–4.0 (2.0)	2.0-8.0 (5.7)	4.0-15.0 (11.1)	9.0-36 (27)	22-88 (66)
Culture volume	100µl	250µl	500µl	1ml	2ml	5ml
K4® Multiplier volume [µl]	1.0	2.5	5.0	10	20	50
Specific multiplying factor for DNA amount	**0.5**	**1.0**	**1.66**	**3.33**	**8.0**	**20.0**
DNA-amount [µg]	0.1-0.25 (0.15)	0.2 -0.5 (0.3)	0.3-0.8 (0.5)	0.7-1.7 (1)	1.6-4.0 (2.4)	4-10 (6)
K4® Transfection Reagent volume [µl]	0.2-1.25 (0.6)	0.4-2.5 (1.2)	0.6-4.0 (2.0)	1.4-8.5 (4.0)	3.2-20 (9.6)	8-50 (24)
Serum-free medium for DNA dilution [µl]	5	15	30	50	130	300
Serum-free medium for K4® Transfection Reagent dilution [µl]	5	15	30	50	130	300

* Cell seeding counts depend on the cell type and must be optimized - ideally by creating a growth curve (see 3.1 Key optimization parameters – Cell seeding counts).

mRNA-Transfection

Proposed starting points in brackets

Culture vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate	60mm plate
Growth area [cm^2]	0.31	1.0	1.9	3.7	9.0	22.0
Cell count of adherent cells seeded [× 10^5]	0.15	0.5	1.0	1.9	4.5	11
Cell count of suspension cells seeded [× 10^5]	0.5	1.5	2.9	5.6	13.5	33
Culture volume	100µl	250µl	500µl	1ml	2ml	5ml
K4® Multiplier volume [µl]	1.0	2.5	5.0	10	20	50
Specific multiplying factor mRNA-amount [µg]	**0.5** 0.1-0.5 (0.2)	**1.0** 0.2-0.9 (0.4)	**1.66** 0.3-1.5 (0.7)	**3.33** 0.7-3.0 (1.3)	**8.0** 1.6-7.0 (3.2)	**20.0** 4-18 (8)
K4® Transfection Reagent volume [µl]	0.2-1.5 (0.4)	0.4-2.7 (0.8)	0.6-4.5 (1.4)	1.4-9.0 (2.6)	3.2-21 (6.4)	8-54 (16)
Serum-free medium for mRNA dilution [µl]	5	15	30	50	130	300
Serum-free medium for K4® Transfection Reagent dilution [µl]	5	15	30	50	130	300

mi/siRNA-Transfection

Proposed starting points in brackets

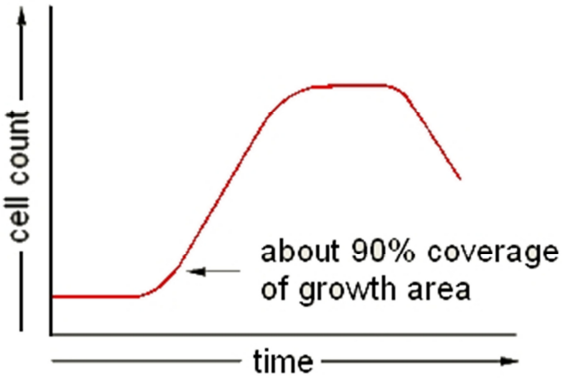
Culture vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate	60mm plate
Growth area [cm^2]	0.31	1.0	1.9	3.7	9.0	22.0
Cell count of adherent cells seeded [× 10^5]	0.15	0.5	1.0	1.9	4.5	11
Cell count of suspension cells seeded [× 10^5]	0.5	1.5	2.9	5.6	13.5	33
Culture volume	100µl	250µl	500µl	1ml	2ml	5ml
K4® Multiplier volume [µl]	1.0	2.5	5.0	10	20	50
Specific multiplying factor RNA-amount [µg] (0.1 µg = ca. 7.5 pmol)	**0.5** 0.1-0.5 (0.2)	**1.0** 0.2-0.9 (0.4)	**1.66** 0.3-1.5 (0.7)	**3.33** 0.7-3.0 (1.3)	**8.0** 1.6-7.0 (3.2)	**20.0** (4-18) (8)
K4® Transfection Reagent volume [µl]	0.2-1.5 (0.4)	0.4-2.7 (0.8)	0.6-4.5 (1.4)	1.4-9.0 (2.6)	3.2-21 (6.4)	8-54 (16)
Serum-free medium for RNA dilution [µl]	5	15	30	50	130	300
Serum-free medium for K4® Transfection Reagent dilution [µl]	5	15	30	50	130	300

3. Explanatory Remarks

3.1 Key optimization parameters

Cell seeding counts

At the time of DNA transfection the cell proliferation rate must be at its highest. The seeded cell count must be adjusted accordingly, ideally using a growth curve.



Typical growing curve of adherent cell.
At the time of the beginning of highest proliferation (=optimal point of transfection)
the growing area is about 90% covered.

Working with a lower cell density requires the reduction of the amount of DNA to avoid toxic effects. In addition the transfection efficiency decreases with reduced proliferation.

The proliferation rate is of minor importance for the transfection of RNA.

Ratio of nucleic acid to transfection reagent

A key variable is the ratio of nucleic acid to transfection reagent. The optimum ratio of nucleic acid – K4® Transfection Reagent must be optimized for each cell type and application.

Quantity of transfection complex

Each cell type has individual characteristic compatibility levels for different lipoplexes. Lipoplex volume must therefore be optimized to the cell count used for each cell type and for each new application of expression product.

Especially for classical suspension cells without extra-cellular matrix (haematopoietic cells or derived cell lines) it is recommended to increase the amount of DNA and K4®Transfection Reagent. The extra-cellular matrix is used by the lipoplexes as an entry gate for endocytosis. If no extra-cellular matrix exists or is only weakly developed this can be compensated by increasing the lipoplex amount.

Serum effects

No negative serum effects in connection with the K4® Transfection System are known to date. In all cases involving **complex formation** it should be noted that serum presence must be avoided as serum significantly impairs the process of complex formation. Once lipoplex formation is completed, serum contact is no longer a factor.

3.2 Troubleshooting

See the Transfection FAQ section on our website at http://www.biontex.com.

4. Miscellaneous

4.1 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.

K4® is a registered trademark of Biontex Laboratories GmbH.

4.2 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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