XpressBio

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Ultra 2K Transfection System

Catalog Number	Size
U2KTS60-0.75	Ultra 2K Transfection Reagent: 1 x 0.75 ml Ultra 2K Multiplier: 1 x 3.25 ml
U2KTS60-1	Ultra 2K Transfection Reagent: 1 x 1.5 ml Ultra 2K Multiplier: 1 x 6.5 ml
U2KTS60-2	Ultra 2K Transfection Reagent: 2 x 1.5 ml Ultra 2K Multiplier: 2 x 6.5 ml
U2KTS60-5	Ultra 2K Transfection Reagent: 5 x 1.5 ml Ultra 2K Multiplier: 5 x 6.5 ml

Description:

Eukaryotic cells respond to foreign substances such as lipopolysaccharides, foreign DNA or RNA, and foreign proteins by taking defensive measures to inhibit invasion of potential pathogens. The cells then send messenger molecules to neighboring cells to implement defensive measures. These defensive mechanisms can greatly impair the transfection process for certain cells.

The Ultra 2K Transfection System is comprised of the optimized Ultra 2K Transfection Reagent based on powerful cationic lipids, and the Ultra 2K Multiplier which decreases the cells' ability to detect foreign nucleic acids, resulting in increased transfection efficiency.

Application: Transfection of nucleic acids into mammalian cells

Assays: 500 – 1850 (48-well) with 1.5 ml Ultra 2K Transfection Reagent

Shipping: Shipped at room temperature.

Storage: +4°C

Stability: See label for expiration date.

Formulations of liposomes like Ultra 2K Transfection Reagent change their size distribution after long storage at +4°C, which can have slight adverse effects on the transfection efficiency. This effect can be reversed by a freeze-thaw cycle. It is recommended to perform a freeze-thaw cycle before first use, and subsequently monthly to yield optimal results.

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

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General Guidelines

Cell Conditions

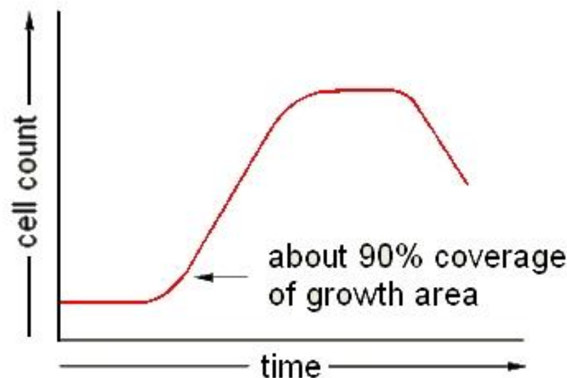
Cells to be transfected should be well proliferating and healthy. Cells which have been in a quiescent state at confluency for a while (before seeding) may not be transfected as efficiently as cells which are growing rapidly. Therefore, it is recommended to use regularly passaged cells for transfection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

Cell Confluency and Seeding Counts

Each cell line has a specific optimal range of amounts for nucleic acids, optimal DNA-lipid ratio, optimal mRNA-lipid ration and optimal Ultra 2K Multiplier concentration related to the number of cells used. The optimal Ultra 2K Multiplier concentration may be different for the transfection of DNA and mRNA.

For DNA transfection, 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 update of DNA into the nucleus, a prerequisite of expression, through the breakup and rebuilding of the core membrane during cell division.

The seeded cell count must be adjusted accordingly, ideally using a growth curve.



Typical growth 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 lower cell density requires the reduction of the amount of nucleic acid and Ultra 2K Multiplier to avoid toxic effects. In addition, the transfection efficiency decreases with reduced proliferation.

Proliferation rate is of minor importance for the transfection of RNA.

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. Before its use in complex formation, DNA

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or RNA should not be stored diluted in medium for much longer than 5 minutes. Adsorption of DNA or RNA in container materials can result in decrease of 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 because of the different rations of plastic surface area to volume of medium in vessels of varying sizes.

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 Ultra 2K Transfection Reagent up to 5 – 10-fold. The extra-cellular matrix is used by the lipoplexes as an entry gate for endocytosis. If no extra-cellular matrix exists or it is only weakly developed this can be compensated for by increasing the lipoplex amount.

Serum Effects

There are no known negative serum effects with the Ultra 2K Transfection System. For cases involving complex formation, serum presence must be avoided since serum significantly impairs the process of complex formation. Once lipoplex formation is complete, contact with serum is no longer a factor.

Transfection Protocol – DNA

Note: For first use it is recommended to use the optimization protocol. For up and down scaling, please see charts at the end of all protocols.

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.
   Note: Cell seeding counts depend on the cell type and must be optimized, ideally by creating a growth curve (see General Guidelines – Cell Confluency and Seeding Counts).
2. Incubate the cells for 24 hours at 37°C in a CO_2 incubator. For adherent cells the surface should be covered 90 – 100%.
   Note: Working with a lower cell density requires the reduction of the amount of DNA and Ultra 2K Multiplier to avoid toxic effects.
3. Bring the DNA and stock solutions for the Ultra 2K Transfection System to room temperature. Agitate gently before use.
4. Two hours prior to adding the lipoplex, pipet 2.5 µl Ultra2K Multiplier into each well of cells to be transfected.

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5. Prepare the following solutions in a polypropylene vessel. **Medium must be pipetted into vessel first** to prevent the reagent and DNA solutions from coming into direct contact with the vessel material.
   Solution A: 0.3 µg of DNA to 15 µl serum medium.
   Solution B: 1.2 µl of Ultra 2K Transfection Reagent to 15 µl serum free medium.
   Note: For successful transfection of classical suspension cells without extra-cellular matrix (e.g. haematopoietic cells or derived cell lines) up to a 10-fold amount of DNA and Ultra 2K Transfection reagent may be necessary.
6. Mix each solution gently by carefully pipetting one time.
7. Add **Solution A to Solution B** and mix gently by pipetting up and down once. Incubate at room temperature for 15 – 20 minutes.
   Note: The DNA solution (Solution A) MUST be added to the Ultra 2K Transfection Reagent solution (Solution B). Do not add Solution B to Solution A.
8. After incubation immediately add the DNA-lipid complex to the cells, mix gently by agitating the cell culture vessel. Incubate at 37°C in a CO_2 incubator.
9. Remove the transfection mixture after 6 - 24 hours and replace with fresh complete growth medium.
10. Test for reporter gene activity between 24 – 48 hours after addition of lipoplex, depending on cell type and promoter activity.

Optimization Protocol for 48-well Format

Use a suitable reporter gene plasmid (e.g. pCMV-βGal, pCMV-Luc, pEGFP, etc.). Ideally, a growth curve is available to determine optimum cell seeding count. 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.
   Note: Cell seeding counts depend on the cell type and must be optimized, ideally by creating a growth curve (see General Guidelines – Cell Confluency and Seeding Counts).
2. Incubate the cells for 24 hours in a CO_2 incubator at 37°C. For adherent cells the surface should then be covered 90 – 100%.
   Note: Working with a lower cell density requires the reduction of the amount of DNA and Ultra 2K Multiplier to avoid toxic effects.
3. Bring the Ultra 2K Transfection Reagent, Ultra 2K Multiplier, and DNA solution to room temperature. Agitate gently before use.
4. Add the Ultra 2K Multiplier to the cells in 48-well plate as follows:
   Rows A & B: No (0.0µl) Ultra 2K Multiplier added.
   Rows C & D: Add 2.5 µl Ultra 2K Multiplier.
   Rows E & F: Add 5.0 µl Ultra 2K Multiplier.
5. Prepare the following solutions in a polypropylene vessel. **Medium must be pipetted into 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 1000 µl serum-free medium.
   Solution B1: Add 9 µl of Ultra 2K Transfection Reagent to 225 µl serum free medium.

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Solution B2: Add 13.5 µl of Ultra 2K Transfection Reagent to 225 µl serum free medium.
Solution B3: Add 18 µl of Ultra 2K Transfection Reagent to 225 µl serum free medium.
Solution B4: Add 22.5 µl of Ultra 2K Transfection Reagent to 225 µl serum free medium.

6. Mix each solution gently by carefully pipetting one time.
7. Add 225 µl **Solution A to Solution B1, B2, B3, and B4.** Mix by gently pipetting up and down once. Incubate at room temperature for 15 – 20 minutes.
   Note: The DNA or RNA solution (Solution A) MUST be added to the Ultra 2K Transfection Reagent solutions (B solutions). Do not add B Solutions to Solution A.
8. After incubation immediately add the DNA-lipid complexes to the cells as follows:

Complex A + B1: 20 µl into well A1, C1 and E1.
30 µl into well A2, C2 and E2.
40 µl into well A3, C3 and E3.
50 µl into well A4, C4 and E4.

Complex A + B2: 20 µl into well A5, C5 and E5.
30 µl into well A6, C6 and E6.
40 µl into well A7, C7 and E7.
50 µl into well A8, C8 and E8.

Complex A + B3: 20 µl into well B1, D1 and F1.
30 µl into well B2, D2 and F2.
40 µl into well B3, D3 and F3.
50 µl into well B4, D4 and F4.

Complex A + B4: 20 µl into well B5, D5 and F5.
30 µl into well B6, D6 and F6.
40 µl into well B7, D7 and F7.
50 µl into well B8, D8 and F8.

Plate map for reference

	1	2	3	4	5	6	7	8
A	A+B1 20 µl	A+B1 30 µl	A+B1 40 µl	A+B1 50 µl	A+B2 20 µl	A+B2 30 µl	A+B2 40 µl	A+B2 50 µl
B	A+B3 20 µl	A+B3 30 µl	A+B3 40 µl	A+B3 50 µl	A+B4 20 µl	A+B4 30 µl	A+B4 40 µl	A+B4 50 µl
C	A+B1 20 µl	A+B1 30 µl	A+B1 40 µl	A+B1 50 µl	A+B2 20 µl	A+B2 30 µl	A+B2 40 µl	A+B2 50 µl
D	A+B3 20 µl	A+B3 30 µl	A+B3 40 µl	A+B3 50 µl	A+B4 20 µl	A+B4 30 µl	A+B4 40 µl	A+B4 50 µl
E	A+B1 20 µl	A+B1 30 µl	A+B1 40 µl	A+B1 50 µl	A+B2 20 µl	A+B2 30 µl	A+B2 40 µl	A+B2 50 µl
F	A+B3 20 µl	A+B3 30 µl	A+B3 40 µl	A+B3 50 µl	A+B4 20 µl	A+B4 30 µl	A+B4 40 µl	A+B4 50 µl

Row A &B: 0.0 µl Ultra 2K Multiplier. Row C & D: 2.5 µl Ultra 2K Multiplier. Row E &F: 5.0 µl Ultra 2K Multiplier.

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

Transfection Protocol – mRNA

Note: For first use it is recommended to use the optimization protocol. For up and down scaling, please see charts at the end of all protocols.

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 hours at 37°C in a CO_2 incubator.
3. Bring the mRNA and stock solutions for the Ultra 2K Transfection System to room temperature. Agitate gently before use.
4. Two hours prior to adding the lipoplex, pipet 1.5 µl Ultra2K Multiplier into each well of cells to be transfected.
5. Prepare the following solutions in a polypropylene vessel. **Medium must be pipetted into vessel first** to prevent the reagent and mRNA solutions from coming into direct contact with the vessel material.
   Solution A: 0.4 µg of mRNA to 15 µl serum free medium.
   Solution B: 0.8 µl of Ultra 2K Transfection Reagent to 15 µl serum free medium.
6. Mix each solution gently by carefully pipetting one time.
7. Add **Solution A to Solution B** and mix gently by pipetting up and down once. Incubate at room temperature for 15 – 20 minutes.
   Note: The DNA solution (Solution A) MUST be added to the Ultra 2K Transfection Reagent solution (Solution B). Do not add Solution B to Solution A.
8. After incubation immediately add the mRNA-lipid complex to the cells, mix gently by agitating the cell culture vessel. Incubate at 37°C in a CO_2 incubator.
9. Remove the transfection mixture after 6 - 24 hours and replace with fresh complete growth medium.
10. Test for reporter gene activity between 24 – 48 hours after addition of lipoplex, depending on cell type and promoter activity.

Optimization Protocol for 48-well Format

Use a suitable mRNA coding for a reporter gene like βGal, 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 hours in a CO_2 incubator at 37°C.
3. Bring the Ultra 2K Transfection Reagent, Ultra 2K Multiplier, and mRNA solution to room temperature. Agitate gently before use.

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4. Add the Ultra 2K Multiplier after the growth phase (approximately 24 hours) to the cells in 48-well plate as follows:
   Rows A & D: No (0.0µl) Ultra 2K Multiplier added.
   Rows B & E: Add 1.5 µl Ultra 2K Multiplier added.
   Rows C & F: Add 3.0 µl Ultra 2K Multiplier.
   Incubate the cells for 2 hours.
5. Prepare the following solutions in a polypropylene vessel. **Medium must be pipetted into vessel first** to prevent the reagent and mRNA solutions from coming into direct contact with the vessel material.
   Solution A: Add 30 µg mRNA to 700 µl serum-free medium.
   Solution B1: Add 30 µl of Ultra 2K Transfection Reagent to 330 µl serum free medium.
   Solution B2: Add 44 µl of Ultra 2K Transfection Reagent to 330 µl serum free medium.
6. Mix each solution gently by carefully pipetting one time.
7. Add 350 µl **Solution A to Solution B1 and B2.** Mix by gently pipetting up and down once. Incubate at room temperature for 15 – 20 minutes.
   Note: The mRNA solution (Solution A) MUST be added to the Ultra 2K Transfection Reagent solutions (B solutions). Do not add B Solutions to Solution A.
8. After incubation immediately add the DNA-lipid complexes to the cells as follows:

Complex A + B1: 10 µl into well A1, B1 and C1.
15 µl into well A2, B2 and C2.
20 µl into well A3, B3 and C3.
25 µl into well A4, B4 and C4.
30 µl into well A5, B5, and C5.
35 µl into well A6, B6, and C6.
40 µl into well A7, B7, and C7.
45 µl into well A8, B8, and C8.

Complex A + B2: 10 µl into well D1, E1 and F1.
15 µl into well D2, E2 and F2.
20 µl into well D3, E3 and F3.
25 µl into well D4, E4 and F4.
30 µl into well D5, E5 and F5.
35 µl into well D6, E6, and F6.
40 µl into well D7, E7, and F7.
45 µl into well D8, E8, and F8.

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

Note: Plate map on following page.

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	1	2	3	4	5	6	7	8
A	A+B1 10 µl	A+B1 15 µl	A+B1 20 µl	A+B1 25 µl	A+B1 30 µl	A+B1 35 µl	A+B1 40 µl	A+B1 45 µl
B	A+B1 10 µl	A+B1 15 µl	A+B1 20 µl	A+B1 25 µl	A+B1 30 µl	A+B1 35 µl	A+B1 40 µl	A+B1 45 µl
C	A+B1 10 µl	A+B1 15 µl	A+B1 20 µl	A+B1 25 µl	A+B1 30 µl	A+B1 35 µl	A+B1 40 µl	A+B1 45 µl
D	A+B2 10 µl	A+B2 15 µl	A+B2 20 µl	A+B2 25 µl	A+B2 30 µl	A+B2 35 µl	A+B2 40 µl	A+B2 45 µl
E	A+B2 10 µl	A+B2 15 µl	A+B2 20 µl	A+B2 25 µl	A+B2 30 µl	A+B2 35 µl	A+B2 40 µl	A+B2 45 µl
F	A+B2 10 µl	A+B2 15 µl	A+B2 20 µl	A+B2 25 µl	A+B2 30 µl	A+B2 35 µl	A+B2 40 µl	A+B2 45 µl

Row A & D: 0.0 µl Ultra 2K Multiplier. Row B & E: 2.5 µl Ultra 2K Multiplier. Row C & F: 5.0 µl Ultra 2K Multiplier.

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

Transfection Protocol – 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 hours at 37°C in a CO_2 incubator.
3. Bring the stock solutions of Ultra 2K Transfection System and RNA to room temperature and agitate gently to ensure they are evenly mixed.
4. Prepare the following solutions in polypropylene vessels. **Medium must be pipetted into 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 Ultra 2K Transfection Reagent to 15 µl serum free medium.
5. Mix each solution by gently pipetting up and down once.
6. Add **Solution A to Solution B** and mix gently by pipetting up and down once. Incubate at room temperature for 15 – 20 minutes.
   Note: The mi/siRNA solution (Solution A) MUST be added to the Ultra 2K Transfection Reagent solution (Solution B). Do not add Solution B to Solution A.

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7. 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.
8. Remove the transfection mixture after 6 – 24 hours and replace with fresh complete growth medium.
9. Test for reporter gene activity between 12 – 72 hours after addition of the lipoplex.

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

Up and Downscale

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.

Note:

- The cell count seeded per well is determined by the optimal cell count/cm^2.
  Note: Cell seeding counts depend on the cell type and must be optimized – ideally by creating a growth curve (see Cell Confluency and Seeding Counts under General Guidelines).
- The concentration of the Ultra 2K Multiplier (µl Ultra 2K Multiplier / µl culture volume) is identical for all culture vessels and results from the optimal value.
- 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 volume. Use the multiplying factors shown in each case.
- The nucleic acid-lipid ratio is the optimal value for all vessels.
- The values in parenthesis correspond to the proposed starting points.

Up and downscaling charts on the following pages.

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DNA Transfection

Proposed starting points for optimization in parenthesis.

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 [$x10^5$]	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 [$\times10^5$]	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	100 µl	250 µl	500 µl	1 ml	2 ml	5 ml
Ultra 2K Multiplier Volume [µl]	0-2 (1.0)	0-5 (2.5)	0-10 (5)	0-20 (10)	0-40 (20)	0-50 (50)
Specific multiplying factor for DNA amount **DNA amount [µg]**	**0.5** 0.1-2.5 (0.15)	**1.0** 0.2-0.5 (0.3)	**1.66** 0.3-0.8 (0.5)	**3.33** 0.7-1.7 (1)	**8.0** 1.6-4.0 (2.4)	**20.0** 4-10 (6)
Ultra 2K 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 Ultra 2K Transfection Reagent dilution [µl]	5	15	30	50	130	300

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mRNA Transfection

Proposed starting points for optimization in parenthesis.

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 [$x10^5$]	0.15	0.5	1.0	1.9	4.5	11
Cell count of suspension cells seeded [$\times10^5$]	0.5	1.5	2.9	5.6	13.5	33
Culture Volume	100 µl	250 µl	500 µl	1 ml	2 ml	5 ml
Ultra 2K Multiplier Volume [µl]	0-1 (0.5)	0-3 (1.5)	0-6.0 (3)	0-12 (6)	0-24 (12)	0-60 (30)
Specific multiplying factor for mRNA amount	**0.5**	**1.0**	**1.66**	**3.33**	**8.0**	**20.0**
mRNA amount [µg]	0.1-0.5 (0.2)	0.2-0.9 (0.4)	0.3-1.5 (0.7)	0.7-3.0 (1.3)	1.6-7.0 (3.2)	4-18 (8)
Ultra 2K Transfection Reagent volume [µl]	0.2-1.25 (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 Ultra 2K Transfection Reagent dilution [µl]	5	15	30	50	130	300

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mi/siRNA Transfection

Proposed starting points for optimization in parenthesis.

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 [x10^5]	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	100 µl	250 µl	500 µl	1 ml	2 ml	5 ml
Ultra 2K Multiplier Volume [µl]	0	0	0	0	0	0
Specific multiplying factor for RNA amount	**0.5**	**1.0**	**1.66**	**3.33**	**8.0**	**20.0**
RNA amount [µg] (0.1 µg=ca. 7.5 pmol)	0.1-0.5 (0.2)	0.2-0.9 (0.4)	0.3-1.5 (0.7)	0.7-3.0 (1.3)	1.6-7.0 (3.2)	4-18 (8)
Ultra 2K Transfection Reagent volume [µl]	0.2-1.25 (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 Ultra 2K Transfection Reagent dilution [µl]	5	15	30	50	130	300