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Ultra 4K Transfection System

Catalog Number	Size
U4KTS80-1	Ultra 4K Transfection Reagent: 1 x 1.0 ml Ultra 4K Multiplier: 1 x 3.5 ml
U4KTS80-2	Ultra 4K Transfection Reagent: 2 x 1.0 ml Ultra 4K Multiplier: 2 x 3.5 ml
U4KTS80-5	Ultra 4K Transfection Reagent: 5 x 1.0 ml Ultra 4K Multiplier: 5 x 3.5 ml

Description:

Eukaryotic cells respond to foreign substances such as lipopolysaccharides, foreign DNA or RNA, and foreign proteins and take defensive measures against invasion by potential pathogens. The cells 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 Ultra 4K Transfection System is comprised of the optimized Ultra 4K Transfection Reagent based on powerful cationic lipids, and the Ultra 4K Multiplier which decreases the cells' ability to detect foreign nucleic acids, resulting in increased transfection efficiency.

The Ultra 4K Transfection System is an advanced development of the Ultra 2K Transfection System.

Application: Transfection of nucleic acids into mammalian cells

Assays: 300 – 1400 (48-well) with 1.0 ml Ultra 4K Transfection Reagent

Shipping: Shipped at room temperature.

Storage: +4°C

Stability: See label for expiration date.

Formulations of liposomes like Ultra 4K 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. **Do not freeze the Ultra 4K Multiplier.**

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

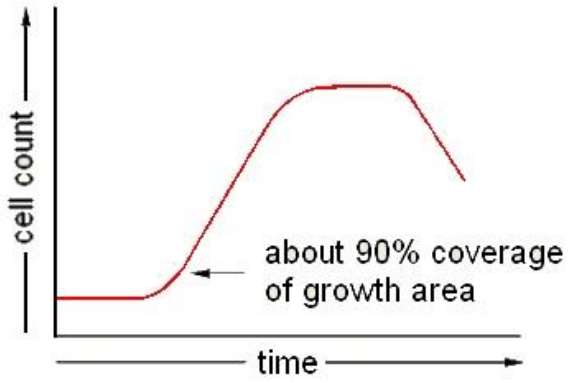
Each cell line has a specific optimal range of amounts for nucleic acids, optimal DNA-lipid ratio and optimal RNA-lipid ration related to the number of cells used.

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.

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

Optimal 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.

At the time of the 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 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.

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Working with lower cell density requires the reduction of the amount of DNA 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. Bacterial related contamination such as endotoxins will significantly impair transfection efficiency.

Adsorption Process

Before forming a complex with the Ultra 4K Transfection Reagent, the DNA or the RNA and the reagent should not be kept in the 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 rations of plastic surface area to volume of medium in vessels of varying sizes.

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 to avoid toxic effects.
3. Bring the DNA and stock solutions for the Ultra 4K Transfection System to room temperature. Agitate gently before use.
4. 30 minutes prior to adding the lipoplex, pipet 2.5 µl Ultra 4K Multiplier (1% related to complete growth medium) 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 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 4K 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 4K Transfection reagent may be necessary.
6. Mix each solution gently by carefully pipetting one time.

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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 4K 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 4K Multiplier to avoid toxic effects.
3. Bring the Ultra 4K Transfection Reagent, Ultra 4K Multiplier, and DNA solution to room temperature. Agitate gently before use.
4. Add 2.5 µl of the Ultra 4K Multiplier (1% related to complete growth medium) to the cells and incubate for 30 minutes.
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 4K Transfection Reagent to 225 µl serum free medium.
   Solution B2: Add 13.5 µl of Ultra 4K Transfection Reagent to 225 µl serum free medium.
   Solution B3: Add 18 µl of Ultra 4K Transfection Reagent to 225 µl serum free medium.
   Solution B4: Add 22.5 µl of Ultra 4K 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 solution (Solution A) MUST be added to the Ultra 4K 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:

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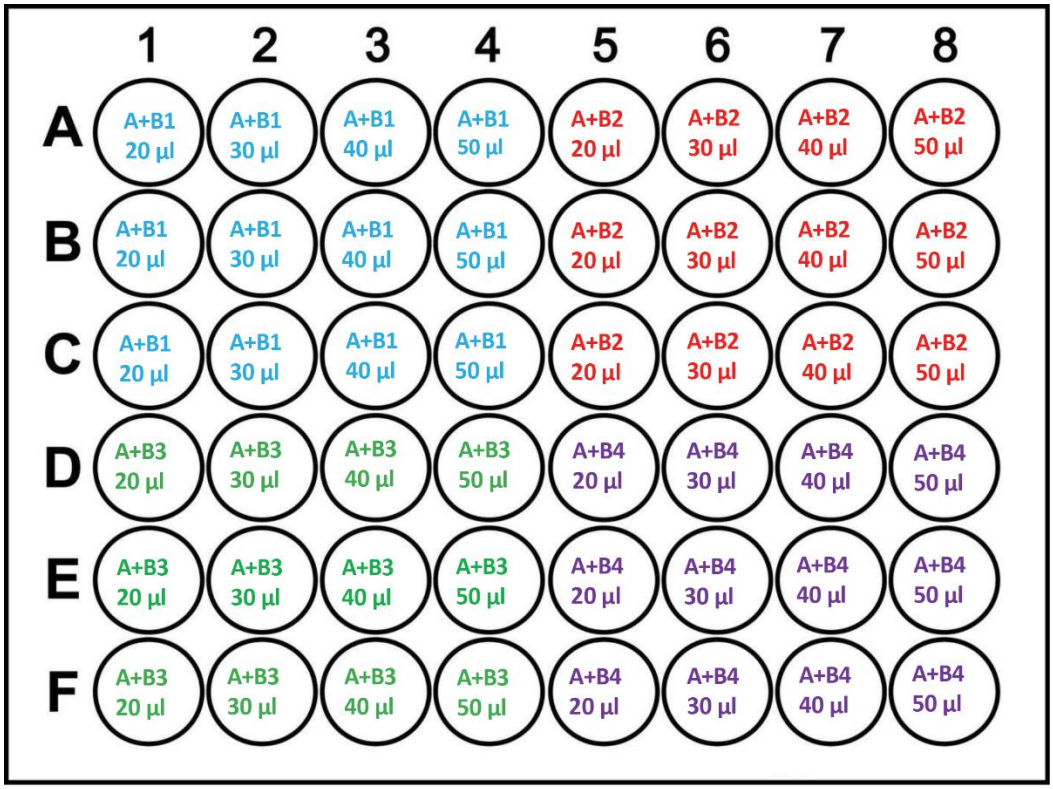
Complex A + B1: 20 µl into well A1, B1 and C1.
30 µl into well A2, B2 and C2.
40 µl into well A3, B3 and C3.
50 µl into well A4, B4 and C4.

Complex A + B2: 20 µl into well A5, B5 and C5.
30 µl into well A6, B6 and C6.
40 µl into well A7, B7 and C7.
50 µl into well A8, B8 and C8.

Complex A + B3: 20 µl into well D1, E1 and F1.
30 µl into well D2, E2 and F2.
40 µl into well D3, E3 and F3.
50 µl into well D4, E4 and F4.

Complex A + B4: 20 µl into well D5, E5 and F5.
30 µl into well D6, E6 and F6.
40 µl into well D7, E7 and F7.
50 µl into well D8, E8 and F8.

Plate map for reference



**2.5 µl of Ultra 4K Multiplier in each well.*

9. Optional: 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.

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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 4K Transfection System to room temperature. Agitate gently before use.
4. 30 minutes prior to adding the lipoplex, pipet 2.5 µl Ultra 4K Multiplier (1% related to complete growth medium) 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 4K 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 4K 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. Optional: 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 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 4K Transfection Reagent, Ultra 4K Multiplier, and mRNA solution to room temperature. Agitate gently before use.
4. 30 minutes before adding the lipoplex, pipet 2.5 µl Ultra 4K Multiplier (1% related to complete growth medium) 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 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 4K Transfection Reagent to 330 µl serum free medium.
   Solution B2: Add 44 µl of Ultra 4K 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 4K 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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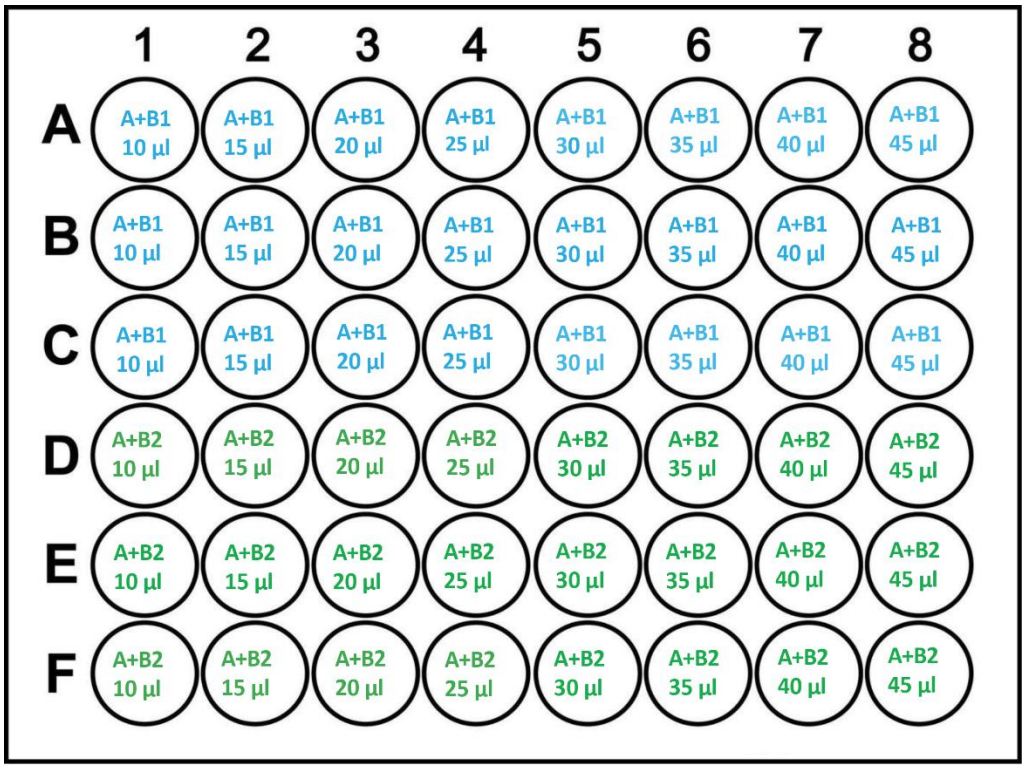

**2.5 µl of Ultra 4K Multiplier in each well.*

9. Optional: 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 4K Transfection System and RNA to room temperature and agitate gently to ensure they are evenly mixed.
4. 30 minutes before adding the lipoplex, pipet 2.5 µl Ultra 4K Multiplier (1% related to complete growth medium) into each well of cells to be transfected.
5. 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 4K Transfection Reagent to 15 µl serum free medium.
6. Mix each solution by gently pipetting up and down once.
7. Add **Solution A to Solution B** and mix gently by pipetting up and down once. Incubate at room temperature for 15 – 20 minutes.

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Note: The mi/siRNA solution (Solution A) MUST be added to the Ultra 4K Transfection Reagent solution (Solution B). Do not add Solution B to Solution A.

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 hours and replace with fresh complete growth medium.
10. 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).
- 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 [×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	100 µl	250 µl	500 µl	1 ml	2 ml	5 ml
Ultra 4K 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]	0.1-2.5 (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)
Ultra 4K 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 4K 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 4K Multiplier Volume [µl]	1.0	2.5	5.0	10	20	50
Specific multiplying factor	**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 4K 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 4K 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 [$\times10^5$]	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 4K Multiplier Volume [µl]	1.0	2.5	5.0	10	20	50
Specific multiplying factor	**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 4K 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 4K Transfection Reagent dilution [µl]	5	15	30	50	130	300