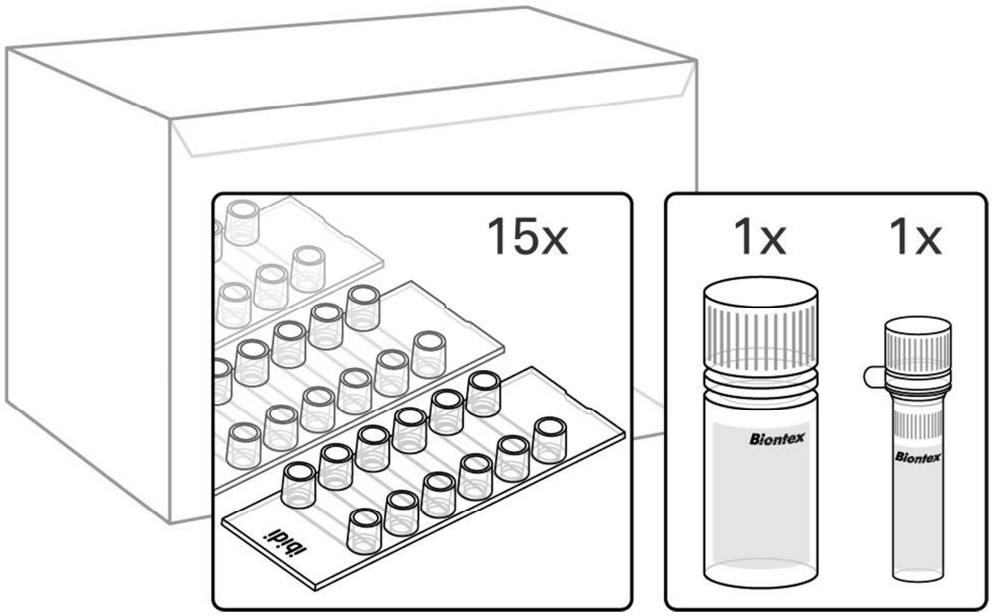
Cell Culture, Transfection and Microscopy in one Slide

The **µ–Transfection Kit VI** combines the efficient transfection of METAFECTENE® µ FluoR (Biontex Laboratories GmbH) with the µ–Slides (ibidi GmbH), designed for cell microscopy. The µ–Transfection Kit VI comprises the transfection reagent METAFECTENE® µ FluoR (Biontex Laboratories GmbH), 1x PBS–Buffer and 15 µ–Slides VI (ibidi GmbH).

METAFECTENE® µ FluoR is a fluorescence–labelled transfection reagent specially elaborated for transfection of mammalian cells in the µ–Slide VI. It offers a low toxicity with excellent transfection results as well as a simple and fast protocol. In combination with the µ–Slide VI you obtain a deeper insight in the processes following the transfection by life cell imaging. The rhodamine label enables the transfection process to be visualized and analysis of lipoplex localization and uptake to be conducted.

The **µ–Slide VI** is designed for highly resolving microscopy and is optimal for experiments both with living cells and for fixation and staining of cells. Due to the high optical quallity of the material fluorescence microscopy is possible with every wavelength and a maximum of resolution.



Contents µ–Transfection Kit VI FluoR:		
METAFECTENE® µ FluoR	100 µl	Biontex Laboratories GmbH
1 x PBS	10 ml	Biontex Laboratories GmbH
µ–Slide VI, ibiTreat	15 x	ibidi GmbH

Shipping:	At room temperature (freeze immediatly on receipt!)	
Storage:	METAFECTENE® µ 1 x PBS µ–Slide VI	≤ -15°C 4°C room temperature
Stability:	Best before (unopened): see label	
Use:	Only for research purposes in vitro, not intended for human or animal diagnostic, therapeutic or other clinical uses.	

Contents

1 General Information **3**
1.1 Working with ibidi µ–Slides . . . 3
1.1.1 Material . . . 3
1.1.2 Geometry of the µ–Slide VI . . . 3
1.1.3 Preparation for Cell Microscopy . . . 3
1.1.4 Filling of the Slides . . . 3
1.1.5 Immersion Oil . . . 3
1.2 Working with METAFECTENE® µ FluoR . . . 4
1.2.1 Specifictions . . . 4
1.2.2 Quality Control . . . 4
1.2.3 Explanatory Remarks . . . 4
1.2.4 Storage . . . 4
1.2.5 State of Cells . . . 4
1.2.6 Quality of Genetic Material . . . 4

2 Working Instructions: DNA Transfection **5**
2.1 Preparing the Reagents . . . 5
2.2 Preparing the Cells . . . 5
2.3 Preparing the Lipoplex . . . 5
2.4 Transfection . . . 5
2.5 Evaluation . . . 5

3 Working Instructions: siRNA Transfection **5**
3.1 Preparing the Reagents . . . 5
3.2 Preparing the Cells . . . 5
3.3 Preparing the Lipoplex . . . 6
3.4 Transfection . . . 6
3.5 Evaluation . . . 7
3.6 Following up Assays . . . 7

4 Miscellaneous **7**
4.1 Tip . . . 7
4.2 Important Information . . . 7
4.3 Warranty . . . 7

1 General Information

1.1 Working with ibidi µ–Slides

1.1.1 Material

The µ–Slides consist of a plastic with highest optical quality. The material exhibits extremely low birefringence and autofluorescence, both similar to that of glass. It is not possible to detach the bottom from the slide. The µ–Slides are not autoclavable since they are temperature stable up to 60°C/140°F only. Please note that gas exchange between the channel and incubator's atmosphere occurs partially through the plastic bottom which should not be covered. Thus, it is recommended to place the µ–Slide on an ibidi µ–Slide rack.

1.1.2 Geometry of the µ–Slide VI

The µ–Slide VI provides standard slide format according to ISO 8037/1. The lateral adapter to adapter distance of 9 mm (like 96 well plates) allows using multichannel pipettes.

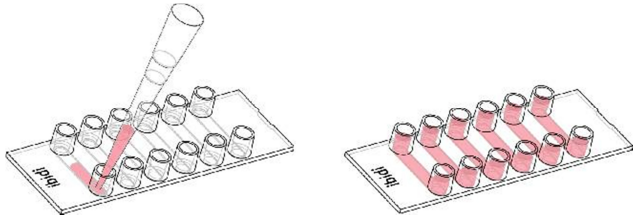
Geometry	
Number of channels	6
Channel volume	30 µl
Channel length	17 mm
Channel width	3.8 mm
Channel height	0.4 mm
Adapters	female Luer
Volume per reservoir	60 µl
Growth area	0.6 cm^2 per channel
Coating area using 30 µl	1.2 cm^2 per channel
Bottom matches coverslip	No. 1.5

1.1.3 Preparation for Cell Microscopy

To analyze your cells no special preparations are necessary. Cells can be observed live or fixed directly in the µ–Slide on an inverted microscope. You can use any fixative of your choice. The µ–Slide material is compatible with a variety of chemicals, e.g. Acetone or Methanol. Further specifications can be found at www.ibidi.com. Due to the thin bottom of only 180 µm, high resolution microscopy is possible.

1.1.4 Filling of the Slides

Apply 30 µl of cell suspension per channel of the µ–Slide–VI with a pipet. To avoid air bubbles put the pipet tip right on the channel inlet and point at the channel as shown in the picture below.



After cell attachment (approx. 4 – 6 hours) fill the reservoirs with 60 µl medium each.

1.1.5 Immersion Oil

When using oil immersion objectives, use only the immersion oils specified in the table. The use of a nonrecommended oil could lead to the damage of the plastic material and the objective.

Company	Product	Ordering number
Zeiss	Immersol 518 F	(Zeiss) 444960
Zeiss	Immersol W 2010	(Zeiss) 444969
Leica	Immersion liquid	(Leica) 11513859

Tip:

The day before seeding the cells we recommend to place the cell medium and the µ–Slide into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time.

Trapped air bubbles can be removed from the channel by inclining the µ–Slide and knocking at one edge.

1.2 Working with METAFECTENE® µ FluoR

1.2.1 Specifications

Application	Transfection of nucleic acids into mammalian cells in µ–Slides VI
Formulation	Cationic lipids with colipids in water, convalently attached rhodamine-labeled
Exc_{max}	557 nm
Emi_{max}	571 nm
Sterility	tested
Cell Culture	tested
Shelf Life	1 year
Storage	≤ -15 °C

1.2.2 Quality Control

Standard transfection assay. Absence of bacterial and fungal contamination is verified using thioglycolate medium.

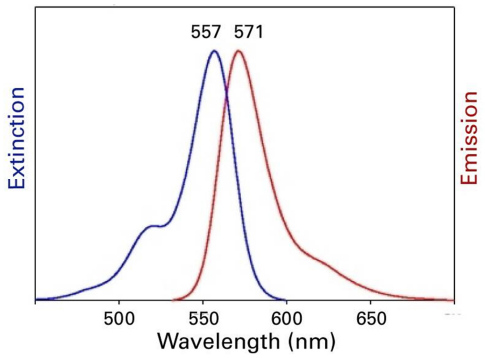
1.2.3 Explanatory Remarks

METAFECTENE® µ FluoR is designed especially for the transfection of mammalian cells in µ–Slides VI. The specific dimensions of the µ–Slides VI growth channels, which account for their excellent optical characteristics, make high demands on the transfection reagent.

Performing a transfection in the µ–Slide VI, effects of diffusion and the adsorptive loss of active transfection complexes and nutrients on the surface play a major role in comparison to conventional cell culture containers.

The composition of the transfection reagent METAFECTENE® µ FluoR and the working protocol are specially adapted to the dimensions and the properties of the µ–Slide VI. This is the only way to obtain optimal transfection results in the µ–Slide VI at excellent optical conditions for microscopy. This is not possible with conventional transfection reagents.

The rhodamine label enables the transfection process to be visualized and analysis of lipoplex localization and uptake to be conducted. In addition, METAFECTENE® µ FluoR can be used in conjunction with appropriately fluorescence–labelled DNA/siRNA to clarify localization of the genetic material introduced, the gene product and the lipoplex.



1.2.4 Storage

METAFECTENE® µ FluoR is delivered non–chilled and should be stored in a freezer at approx. ≤ -15 °C immediately after receipt. Freezing minimizes the natural ageing process of the liposomes.

Storage for several days at room temperature is not a problem provided that the reagent is subsequently stored again at ≤ -15 °C; the number of times the product is thawed for use and refrozen is irrelevant.

1x PBS (Phosphate Buffered Saline) should be stored at 4 °C.

1.2.5 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 (30 – 60% true confluency). Therefore it is recommended to use regularly passaged cells for transfection experiments. Microbial contamination, for example with mycoplasma or fungi, can drastically alter transfection results.

1.2.6 Quality of the Genetic Material

To achieve the best transfection results, the DNA/siRNA should be as pure as possible. Transfection efficiency is significantly reduced by the presence of endotoxins, for example.

2 Working Instructions: DNA Transfection

2.1 Preparing the Reagents

Prior to transfection, bring 1x PBS, METAFECTENE® µ FluoR and the DNA solution to room temperature. Mix each solution gently.

Note: Shear forces may destroy both the lipoplex and free DNA!

2.2 Preparing the Cells

Prepare 100 µl of cell suspension at a concentration of 3 – 4 · 10^5 cells/ml in complete culture medium. This volume is sufficient to fill three channels.

2.3 Preparing the Lipoplex

1. Pipette 1 µl METAFECTENE® µ FluoR in 20 µl 1x PBS and mix the solution by gently pipetting up and down. Add 1 µg DNA by pipetting, and gently mix the entire solution. Use polypropylene (PP) tubes if possible.
2. Incubate the solution at room temperature for 15 min.

2.4 Transfection

1. Add the lipoplex solution to the 100 µl cell suspension and mix gently.
2. Immediately fill each channel with 30 µl of this transfected cell suspension.
3. After cell growth has commenced (approx. 4 – 6 h) fill each reservoir with 60 µl of complete culture medium, then incubate the µ–Slide VI under the normal conditions for the cell line used (e.g. 37°C in atmosphere containing CO_2).

Note: Medium must be changed daily in assays with a duration of over 24 h.

2.5 Evaluation

Evaluation generally takes place 24 – 48 h later. For assays with longer duration (up to 96 h), initial cell density may be reduced to avoid obtaining an excessively confluent culture. To do this, dilute the transfected cell suspension 1:1 with complete culture medium, incubate for 15 min and use to fill the channels as described in section 2.4.

Fluorescence microscopy takes place using a suitable rhodamine filter.

3 Working Instructions: siRNA Transfection

The following instructions for siRNA transfection using µ–Slides VI were designed to deliver optimum knock–down from the first assay. Two different cell concentrations ensure that both the assay duration (24 – 96 h) and the growth speed of the cell lines utilized are extensively covered. In addition, three different lipoplex concentrations ensure that even highly sensitive cell lines can be transfected successfully.

The chart shows an overview of the six solutions to be prepared (the figures refer to the numbering of the tubes given in section 3.3).

Transfection–Parameters		
	4 · 10^5 Zellen/ml	2 · 10^5 Zellen/ml
Lipoplex 1/1	**(A)**	**(D)**
Lipoplex 1/2	**(B)**	**(E)**
Lipoplex 1/4	**(C)**	**(F)**

3.1 Preparing the Reagents

Prior to transfection, bring 1x PBS, METAFECTENE® µ FluoR and the siRNA solution to room temperature. Mix each solution gently.

3.2 Preparing the Cells

Prepare at least 400 µl cell suspension with cell concentration of 3 – 4 · 10^5 cells/ml in complete culture medium.

3.3 Preparing the Lipoplex

1. Six tubes numbered **(A)** to **(F)** are needed. Use polypropylene (PP) tubes if possible.

   Place 40 µl 1x PBS in tube **(A)** and add 2 µl of METAFECTENE® µ FluoR by means of a pipette. Mix the solutions by gently pipetting up and down. Add 2 µg siRNA by pipetting, and gently mix the entire solution.

   Adjust the pipette at half the volume of tube **(A)**. Now place 1x PBS in tube **(B)** and **(C)** by means of a pipette with that setting.

2. Furthermore transfer half the volume from tube **(A)** to tube **(B)** with the above setting and mix gently.

3. Again transfer half the volume from tube **(B)** to tube **(C)** and mix gently as before.

   Incubate the solution at room temperature for 15 min.

3.4 Transfection

4. At the end of the incubation period fill each tube **(D)**, **(E)** and **(F)** with 50 µl complete medium.

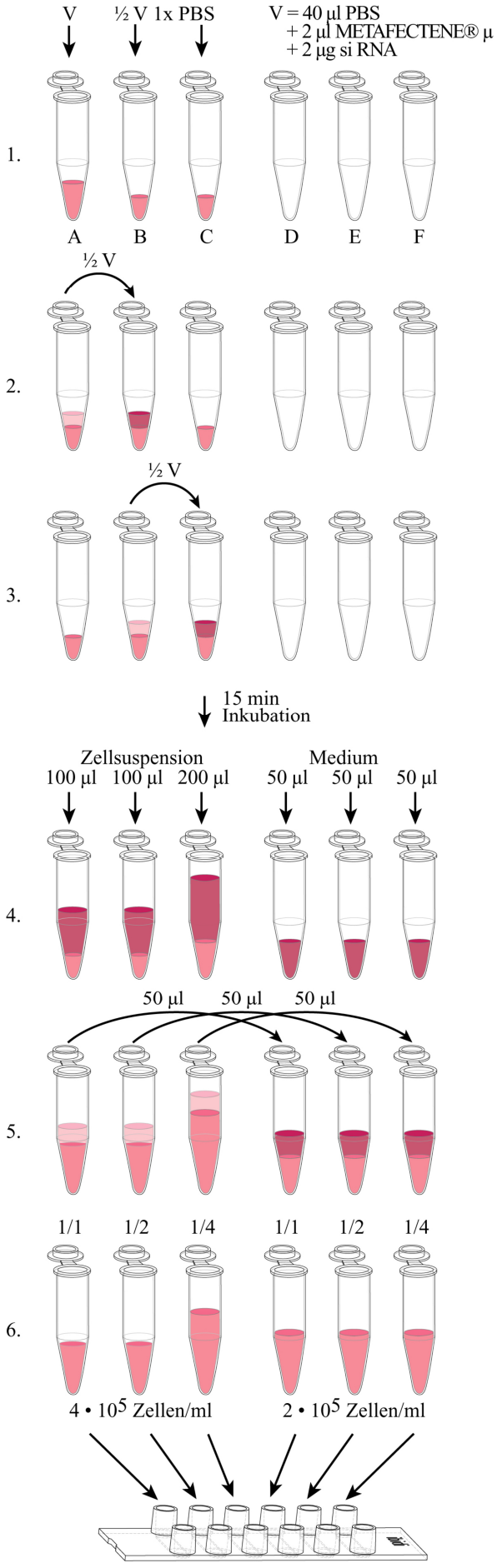
   Immediately after incubation, place 100 µl of cell suspension in each tube **(A)** and **(B)** and 200 µl of cell suspension in tube **(C)**. Mix all solutions gently by pipetting up and down once.

5. Then pipette 50 µl of the solution from tube **(A)** to the medium in tube **(D)**. Similarly, pipette 50 µl from tube **(B)** to tube **(E)** and from tube **(C)** to tube **(F)**. Mix all solutions gently by pipetting up and down once.

6. Now place 30 µl from each of the six tubes in one channel each of a µ–Slide VI.

   After cell attachment (approx. 4 – 6 h) fill each reservoir with 60 µl of complete culture medium, then incubate the µ–Slide VI under the normal conditions for the cell line used (e.g. 37°C in atmosphere containing CO_2).

Note: Medium must be changed daily in assays with a duration of over 24 h.



3.5 Evaluation

Evaluation takes place 24 – 96 h later.

Note: For proteins with extra–long half–life (e.g. GFP at $t_{1/2}$ = 26 h in mammalian cells), do not read off the knock–down result until after approx. 72 hours have elapsed!

Fluorescence microscopy takes place using a suitable rhodamine filter.

3.6 Follow–up assays

Once the optimum parameters of cell and lipoplex concentration have been determined after an assay using the protocol given, further assays can be conducted (using the same cell line and knock–down protein) applying these parameters exclusively.

4 Miscellaneous

4.1 Tip

Highly sensitive cells may benefit from a complete change of medium approx. 6 – 8 hours after cell cultivation begins.

If the cell morphology is considerably worsened after the transfection (e.g. due to a strong interaction of the expressed protein with the cellular metabolism), a reduction of the amount of lipoplex is an option. Keep the ratio of genetic material to METAFECTENE® µ (1 µg : 1µl) con-stant!

4.2 Important Information

METAFECTENE® µ FluoR is a registered trademark of Biontex Laboratories GmbH. Biontex METAFECTENE® µ FluoR is developed and sold for research purposes and in–vitro use only. It is not intended for human therapeutic or diagnostic purposes. Appropriate care should be exercised when handling many of the reagents described in this publication.

4.3 Warranty

Biontex guarantees the performance of the transfection reagent METAFECTENE® µ FluoR, when used in accordance with the information given in this publication, for a period of 12 months from the date of purchase. If you are not completely satisfied with the performance of the product please contact Biontex or one of its authorized distributors.

Biontex Laboratories GmbH
Landsberger Str. 234
80867 München/Laim
Tel: +49 (0)89 3247995-0
Fax: +49 (0)89 3247995-2
Fax2: +49 (0)89 32385994
E-Mail: contact@biontex.com
Internet: www.biontex.com

ibidi GmbH
Am Klopferspitz 19
82152 Planegg/Martinsried
Tel: +49 (0)89 5204617-0
Tel2: +49 (0)89 5204617-31
Fax: +49 (0)89 5204617-59
E-Mail: info@ibidi.de
Internet: www.ibidi.com