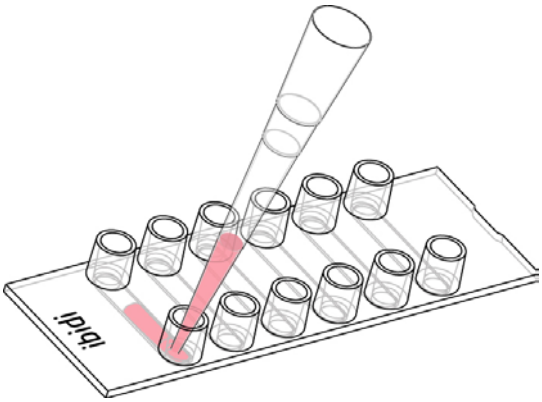
Protocol for Cell Transfection in ibidi µ-Slides

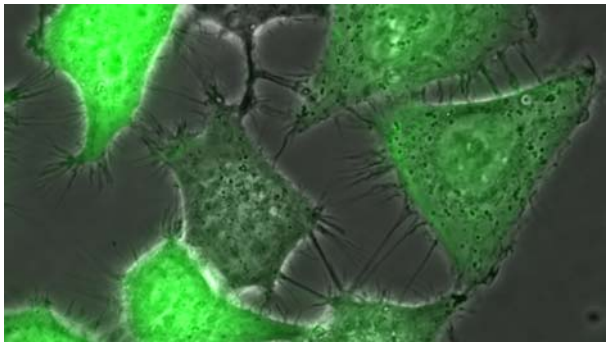
Due to the broad spectrum of transfection methods, this application note only describes an example of DNA transfection conducted in µ-Slides VI $^{0.4}$. Any other transfection protocol may be adapted in a similar way to the handling in ibidi µ-Slides.

The transfection reagent *Metafectene µ FluoR* applied in this protocol provides the possibility of a direct tracking of the reagent uptake by the cells. It is a lipid labeled with a fluorescence tag (rhodamine) which can be detected by fluorescence microscopy.

Principle:
In the first step the plasmid attaches to the lipid which results in a so called lipoplex. Subsequently the lipoplex is added to the cell suspension where it is taken up by endocytosis. In the cell lumen the DNA is released from the lipoplex and is taken up into the cell nucleus at the next cell division.



ibidi µ-Slide VI $^{0.4}$



Cells transfected with GFP in µ-Slide VI $^{0.4}$

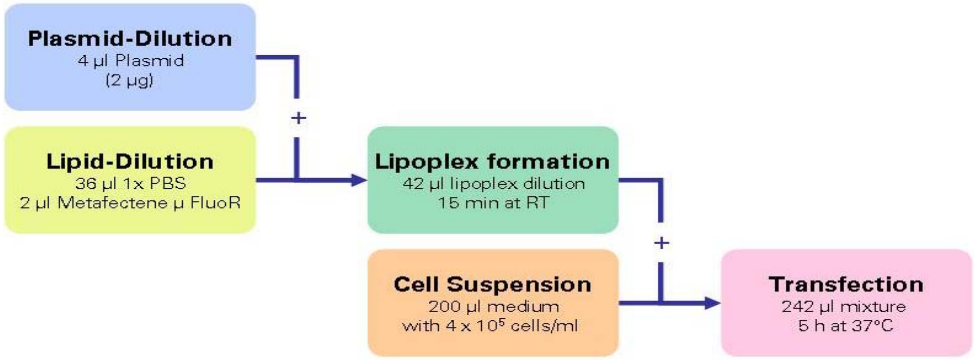
Material	
Cells:	HepG2
Medium:	DMEM 10% FCS
Buffer:	PBS (1x)
Transfection reagent:	Metafectene µ FluoR (Biontex Laboratories GmbH)
Plasmid:	pCMV-GFP (0.5 mg/ml)
Reaction tubes:	polypropylene reaction tubes
Slides:	µ-Slide VI $^{0.4}$, ibiTreat

Preparations one day in advance:

- Put the slides overnight in the incubator to ensure the plastic is prewarmed and the gas is exchanged entirely.
- Equilibrate the medium for the cells in the same way. Place a small vessel into the incubator and leave the cap slightly ajar to make leak the excess pressure.

Preparation of transfection solution:

- Warm up transfection reagent, plasmid solution and PBS buffer to room temperature.
- Dilute 2 µl of the transfection reagent (Metafectene µ FluoR) in 36 µl 1x PBS and mix by gently pipetting up and down.
- Add 4 µl of your plasmid solution to the reagents dilution and mix gently by pipetting up and down.
- For constitution of the lipoplex allow the mixture to stand for 15 minutes at room temeprature. In the meantime prepare the cell suspension.



Preparation of cell suspension:

- Detach and count your cells as usual. Adjust a cell suspension of 4 x 10^5 cells/ml in DMEM. For filling six channels of the µ-Slide VI prepare 200 µl of cell suspension.

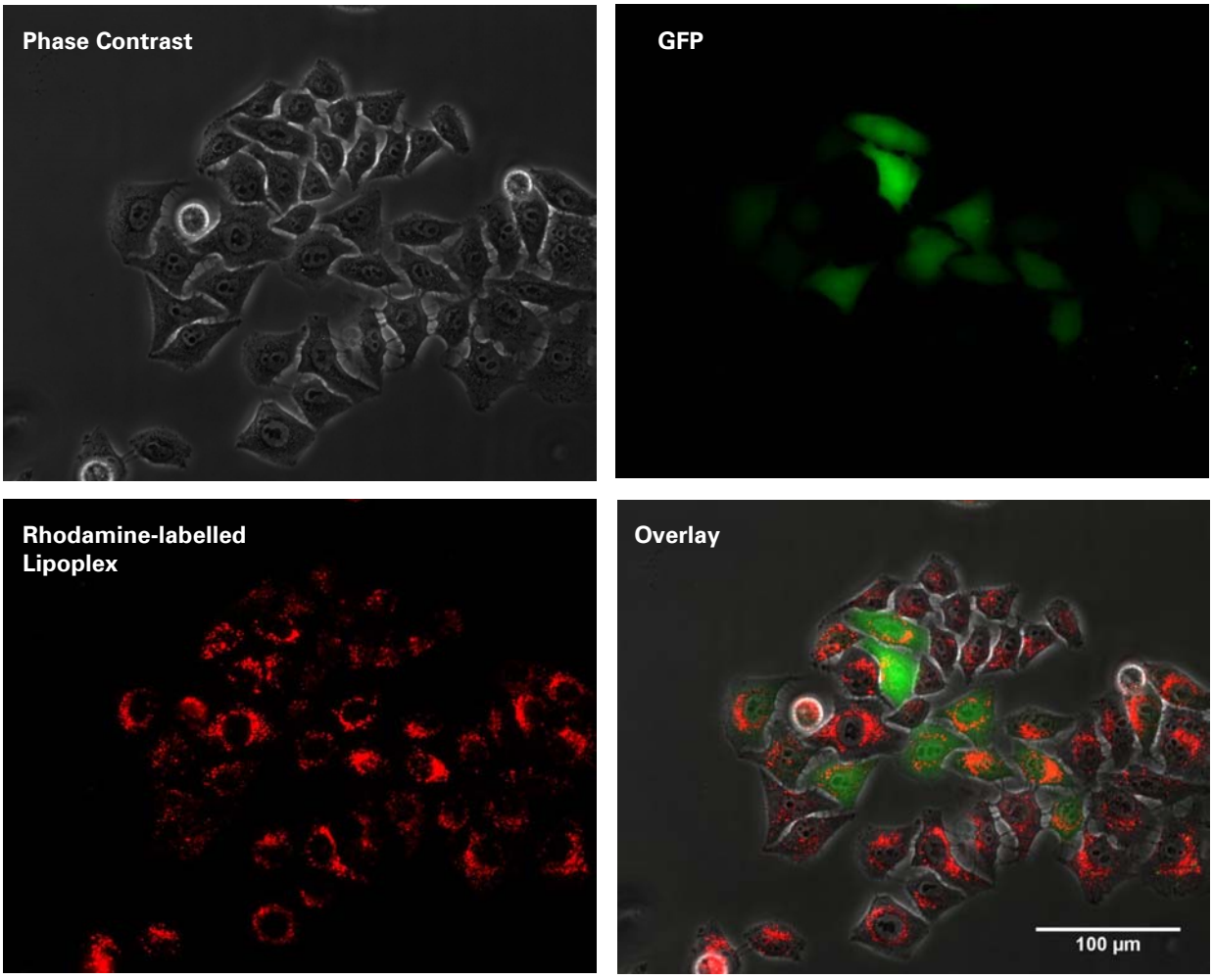
Transfection:

- Add the lipoplex (42 µl) solution the cell suspension (200 µl) and again mix gently with the pipet.
- Fill 30 µl of this mixture into each of the channels and incubate the cells for five hours at 37°C and 5% CO_2. Make sure that evaporation is minimized. Eventually put the µ-Slide into an extra Petri dish with a water soaked wipe.
- During incubation time you can observe the lipoplex uptake by live cell imaging on an inverted microscope.
- After five hours check your cells on a microscope. They should have adhered well.
- Exchange the medium with fresh medium: Put 120 µl of fresh medium into one of the channels reservoirs. The medium will flush through the channel to the opposite reservoir. Aspirate 120 µl with a pipet from the opposite reservoir. Avoid aspirating the medium directly from the channel's inlet!
- Add 120 µl fresh medium to the first reservoir and observe your cells.

Observation of the transfected cells

At this point of the protocol the cells have taken up the lipoplex with the plasmid DNA. For a successful transfection the lipoplex is set free from the endocytotic vesicle and is transported into the cell nucleus. This mostly happens during cell division when the nuclear envelope breaks down.

For observation with time lapse microscopy put the slide on an inverted microscope equipped with an onstage incubation system (e.g. ibidi heating system). Take a picture every 10 minutes. Use a filter set suitable for GFP to visualize the expression of the transfected DNA and a filter set for rhodamine to trace the arrangement of lipoplexes.
In addition to the fluorescence recording you may also record phase contrast pictures. You will see how cells are dividing and afterwards start to express GFP.



HepG2 cell line transfected with GFP: The transfection reagent is labelled with rhodamine. It is visible as small red conglomerates in the cell lumen. Transfected cells produce GFP, which is diffused in the whole cytosol, glowing green.
Pictures were taken with a 20x objective on a Nikon Ti*Eclipse,* 24 hours after adding the lipoplex to the cells.

Watch the movie on www.ibidi.com !

Factors of impact

Concerning cell transfection, there is no principal difference between a µ-Slide and a standard vessel like e.g. a 6 well plate. Hence, all rules for best transfection results apply for both substrates. Nevertheless, we would like to give you some hints derived from our own experience.

Once you have chosen the best transfection method for your cells, transfection efficiencies may still vary from 0% to near 100%, which depends on different experimental factors:

a) Reagent concentration: Since all transfection reagents are more or less toxic, you have to find out which is the maximum concentration that is supported by your cells without a significant mortality rate (should be < 10%). This is very often the most important parameter.

b) Incubation time: If cell mortality is a problem, try to incubate the cells the shortest possible time the reagent's manual recommends. If you find low yields in healthy cells, extend the incubation time to the maximum.

c) Plasmid concentration: There is a connection between plasmid concentration and transfection efficiency. However, this parameter's impact is usually low as long as you follow standard protocols.

d) Preincubation time for Plasmid-Reagent complex formation: Only recommended as a fine-tune parameter. Usually you are best advised when you follow the average times from the reagent's manual.

e) Plasmid purity: a crucial parameter for transfection success. However, sufficient purities are easily achieved with all common plasmid purification kits. Therefore, this is usually not a source of trouble.