

How to do a Cell Transfection in ibidi μ -Slides

Due to the broad spectrum of transfection methods, we only describe an example procedure to illustrate how the manufacturer's instructions or other standard protocols can be adapted to ibidi's μ -Slides.

Cell Transfection with Lipofectamine 2000 (GIBCO)

Plasmid concentration: 1 $\mu\text{g}/\mu\text{l}$

Transfection reagent: Lipofectamine 2000 in OptiMEM

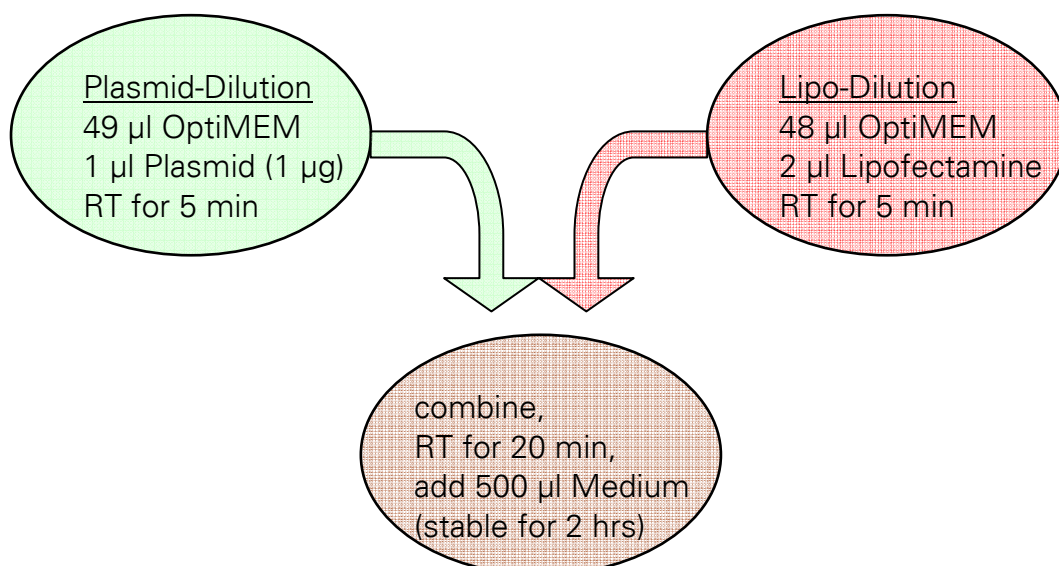
1) Seeding cells

Detach and count your cells as usual. Adjust suspension density to $3\text{-}10 \cdot 10^5$ cells/ml, depending on your cell type. Fill 5 ibidi μ -Slides I (mind the appropriate coating, e.g. collagen, see ibidi catalogue) with 100 μl suspension each and keep them in your incubator over night to obtain a lawn of about 80% confluency.

Don't use antibiotics like pen/strep in your medium since this would impair your transfection efficiency.

2) Preparation of transfection solution:

Plasmid and Lipofectamine are diluted in OptiMEM in separate tubes. After 5 min at room temperature (RT), these dilutions are combined and allowed to incubate at RT for about 20 min. Upon addition of 500 μl full medium (without antibiotics!) the transfection solution is ready for use.



Application Note 07

3) Transfection

Carefully aspirate the medium from the μ -Slides and replace with 100 μ l transfection solution. Leave in your incubator for about 8 hrs.

4) Medium change

Carefully aspirate the transfection solution from the μ -Slides and replace with fresh medium (with or without antibiotics). Protein expression usually peaks from 24 hrs to 2 days after transfection.

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.