

The ibidi product family comprises a variety of different shapes of μ -Slides and μ -Dishes which all have been designed for high-end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.

The μ -Slide upright^{0.8} is specially designed for experimental setups with upright microscopy. A cover glass window, which is inserted on the channel's upper side allows high quality imaging with the objective located above the slide. Nevertheless it is suitable for inverted microscopes as well. The channel format of the slide is applicable in static or perfusion assays. The female Luers allow easy connections to tubing and pump systems.

Material

ibidi μ -Slides and μ -Dishes 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 upper part. The μ -Slides and μ -Dishes are not autoclavable since they are temperature stable up to 80°C/175°F only. Please note that gas exchange between the medium and incubator's atmosphere occurs partially through the plastic bottom which should not be covered.

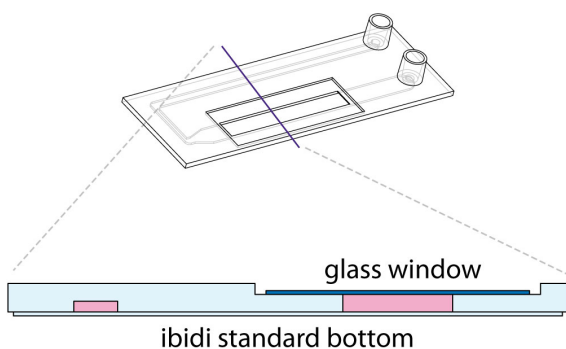
Optical properties ibidi standard bottom

Refractive index n_D (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 μ m)
Material	microscopy plastic

The glass window consists of a glass coverslip with high optical quality. It is not possible to detach the glass part.

Optical properties glass window

Refractive index n_D	1.523
Abbe number	55
Thickness	No. 1.5 (selected quality 170 μ m, \pm 10 μ m)
Material	Schott borosilicate glass, D 263M



Geometry of the μ -Slide upright^{0.8}

The μ -Slide upright^{0.8} provides standard slide format according to ISO 8037/1. The bottom matches coverslip No. 1.5.

Dimensions

Number of channels	1
Total volume without reservoirs	270 μ l
Cell seeding volume	200 μ l
Height of observation window	0.8 mm
Length of observation window	30.7 mm
Width of observation window	5 mm
Growth area of observation area	1.53 cm ²
Coating area using 270 μ l	6 cm ²
Adapters	female Luer
Volume per reservoir	60 μ l

Surface and coating

The μ -Slide is available with ibiTreat and uncoated surface. The ibiTreat surface is a physical treatment and optimized for adhesion of most cell types. Many cell lines as well as primary cells were tested for good cell growth. Only the observation window area is ibiTreat. The uncoated surface is very hydrophobic and allows no direct cell growth. It is suitable for specific coatings or suspension cells.

The surface of the glass coverslip is always identical, no matter if an ibiTreat or uncoated μ -Slide is used.

Coating your μ -Slide upright^{0.8}

The uncoated μ -Slide must be coated to promote cell adhesion. If you like to establish a certain coating for your demands we recommend to test your coating procedure on uncoated and ibiTreat μ -Slides, since we have observed

that some biomolecules adhere differently to hydrophobic or hydrophilic plastic surfaces.

When working with adherent cells attached to the glass window, washing steps (e.g. with PBS) before cell seeding can remove glass dust which is advantageous for direct cell growth on the glass surface. Protein coatings on glass support direct cell growth of adherent cells .

- Prepare your coating solution according to the manufacturer's specifications or reference.
- Apply 270 μl to the channel using a syringe. Leave at room temperature for at least 30 minutes.
- Aspirate the solution and wash with 1 ml ultra-pure water. Let dry at room temperature.

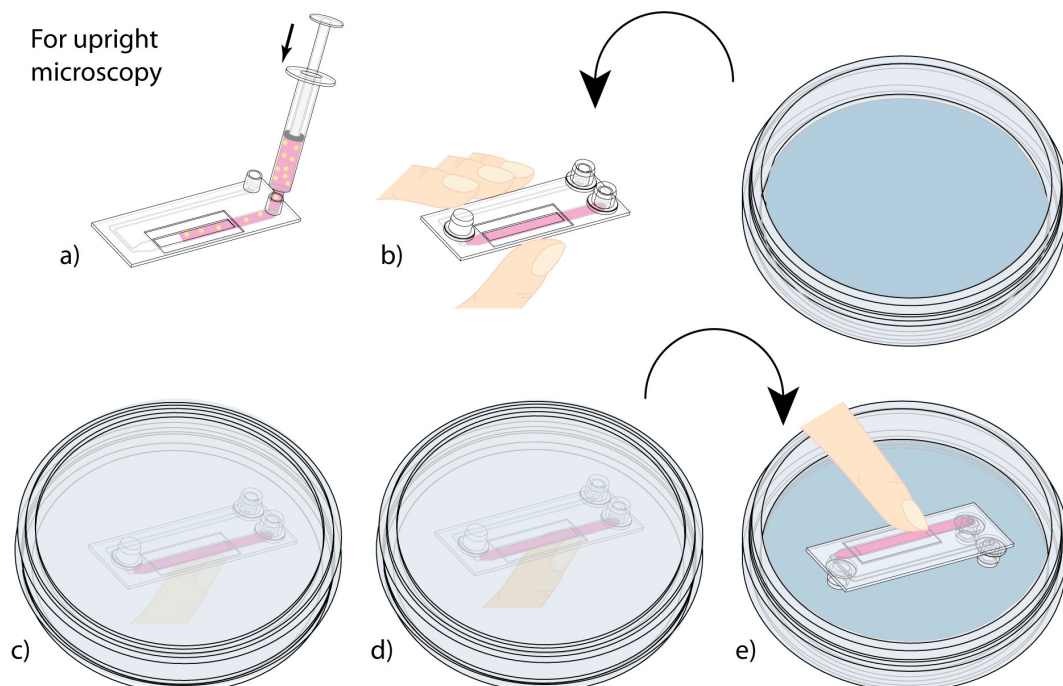
Further information about coatings are provided in [Application Note 08 "Cell culture coating"](#).

Cell seeding for upright microscopy

Important!

For upright microscopy cells are seeded onto the ceiling of the channel. The slide has to be reversed during cell adhesion. It is recommended to seed the cells only to the wider channel part of the slide.

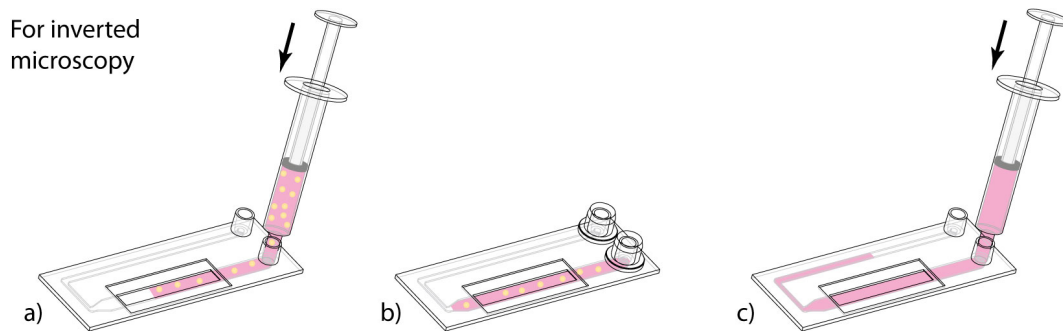
- Trypsinize and count cells as usual. Adjust the cell suspension to $3 - 8 \times 10^5$ cells/ml, depending on your cells. The recommended cell concentration should result in a 50 % optical confluence layer after 24h.
- Fill the cell suspension in a sterile syringe and apply 200 μl to the observation field channel (see figure a).
- Cautiously remove the syringe.
- Flip the slide around "reservoir-side down": Put the three caps onto the slide as shown in figure b. Turn a petri dish over the slide and switch the slide back around together with the petri dish (figure c-e).
- Incubate at 37°C and 5 % CO₂ for cell attachment (30 min – several hours).
- After cell attachment the slide can be flipped back to the normal orientation "reservoir-side up". Fill a sterile syringe with fresh medium and adapt it to the observation channel Luer without including air bubbles. Slowly flush the medium into the channel until the other reservoir is filled completely.
- Cautiously remove the syringe. Leave both reservoirs filled with 60 μl medium for further incubation. Cover reservoirs with the supplied caps.
- The μ-Slide is now ready for applying flow conditions on the adherent cells. For detailed information setting up flow experiments please refer to [Application Note 13 "HUVECs under perfusion"](#).



Cell seeding for inverted microscopy

- Trypsinize and count cells as usual. Adjust the cell suspension to $3 - 8 \times 10^5$ cells/ml, depending on your cells. The recommended cell concentration should result in a 50 % optical confluence layer after 24h.
- Fill the cell suspension in a sterile syringe and apply 200 μl to the observation field channel (see figure a).
- Cautiously remove the syringe.
- Cover reservoirs with the supplied caps.

- Incubate at 37°C and 5 % CO₂ for cell attachment (30 min – several hours).
- After cell attachment fill a sterile syringe with fresh medium and adapt it to the observation channel Luer. Slowly flush the medium into the channel until the other reservoir is filled completely.
- Cautiously remove the syringe. Leave both reservoirs filled with 60 μl medium for further incubation.
- The μ-Slide is now ready for applying flow conditions on the adherent cells. For detailed information setting up flow experiments please refer to [Application Note 13 "HUVECs under perfusion"](#).



Medium exchange

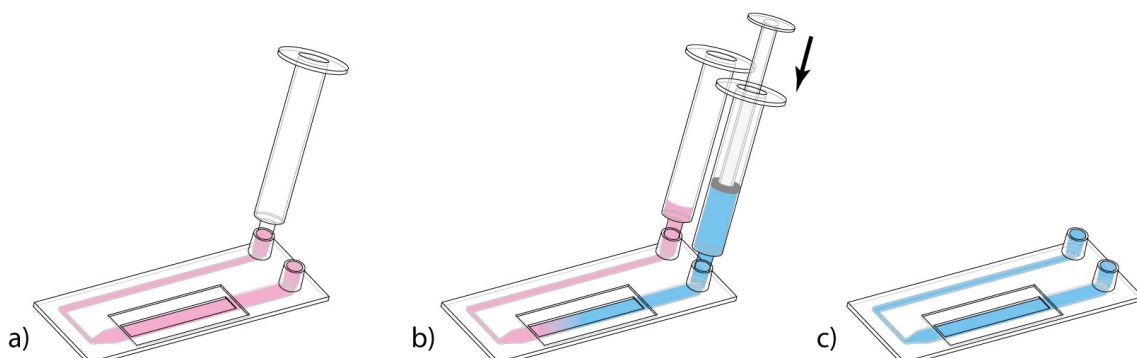
Depending on the cells we recommend exchanging the medium every day in static culture:

- Extract the plunger from a sterile syringe and adapt the syringe to one Luer adapter (figure a).
- Fill a sterile syringe with fresh medium.
- Adapt the syringe to the other Luer adapter without including air bubbles.
- Slowly flush at least 600 μl through the channel.
- Carefully remove the syringe with the old medium and then the syringe with the fresh medium.

- Leave both reservoirs filled with approx. 60 μl each.

Tip:

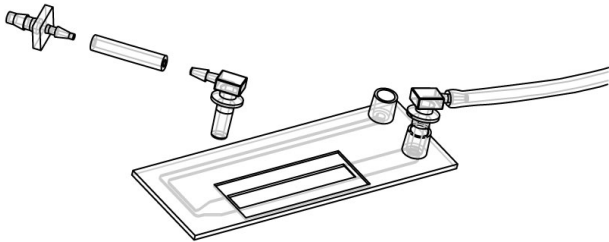
The day before seeding the cells we recommend placing the cell medium, the μ-Slide, and the tubing into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time. For long term analysis of cells under flow conditions we recommend using μ-Slides with ibiTreat as surface.



Flow application

Detailed information about flow rates, shear stress, and shear rates is provided in [Application Note 11 "Shear stress and shear rates"](#) on www.ibidi.com.

Suitable flow kits (μ-Slide upright^{0.8} + tubing and adapters) are also available.



Please contact us for recommended perfusion setups. ibidi provides a variety of pump systems.

Preparation for cell microscopy

As a special feature μ-Slide upright^{0.8} can be used both with upright or inverted microscopes. The reservoir positions are optimized for access with upright objectives. Observe your cells in the glass covered observation area. To

analyze your cells no special preparations are necessary. Cells can be observed live or fixed directly in the μ-Slide. 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.

Immersion oil

When using oil immersion objectives, only the immersion oils specified in the table may be used. The use of different oil can lead to damages of the plastic material and the objective.

Company	Product	Ordering number
Cargille	type DF, Code: 1261	(Cargille) 16242
Cargille	type DF37, Code: 0383	(Cargille) 16239
Cargille	Series AAA, n = 1.330	(Cargille)
Cargille	Series AAA, n = 1.335	(Cargille)
ibidi	immersion oil	(ibidi) 50101
Olympus	8 ml	(Olympus) 035520
Zeiss	518 F	(Zeiss) 444960

μ-Slide upright^{0.8} family

The μ-Slide upright^{0.8} family is available with different surfaces. See table below for choosing your μ-Slide upright^{0.8}.

Ordering number	Treatment or Coating	Characteristics
80226	ibiTreat, tissue culture treated, sterile	hydrophilic plastic – glass window
80221	uncoated, sterile	hydrophobic plastic – glass window

For research use only!

Further technical specifications can be found at www.ibidi.com. For questions and suggestions please contact us by e-mail info@ibidi.de or by telephone +49 (0)89/520 4617 0. All products are developed and produced in Germany.

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