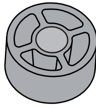
cells in focus ibidi

micro-Insert 3D

Instruction Manual



The micro-Insert 3D is a biocompatible, silicone insert used for creating cellular interfaces on or in gel matrices. Applications include co-cultivation, transport studies, apical-basal cell polarity assays and other hydrogel-based cellular assays.

This document applies to the following products:

80496	**micro-Insert 3D in µ-Dish 35 mm, high ibiTreat**
80499	**25 micro-Inserts 3D for self-insertion**

Material

The micro-Insert 3D is manufactured from biocompatible silicone. Although, the material is autoclavable and compatible to alcohols, it is intended for one-time use only.

NOTE – When using an ibidi µ-Dish, µ-Slide or µ-Plate, make sure that the ibidi Polymer Coverslip is compatible with the immersion oil you intend to use. See page 4 for the list of compatible oils.

Shipping and Storage

This product is sterilized and sealed in a gas-permeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is outlined in the following table.

Conditions	
Shipping conditions	Ambient
Storage conditions	RT (15–25 °C)

Shelf Life	
micro-Inserts	36 months

Surface and Handling

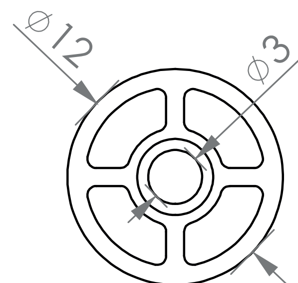
The micro-Inserts can be transferred to any flat, clean, and dry surface. Use sterile tweezers for transfer and gently push the insert in place. Note that only the bottom side is sticky. The micro-Insert 3D does not work on wet or damp surfaces. Uneven or dusty surfaces might also be problematic.

NOTE – Check whether the micro-Insert is completely attached to the surface. If not, gently push the Insert.

Geometry of the micro-Insert 3D

Specifications	
Diameter insert	12 mm
Height insert	6 mm
Diameter inner well	3 mm
Volume inner well	25 µl
Volume outer well	250 µl
Volume gel matrix*	20 µl
Height gel matrix*	0.8 mm
Growth area on gel	0.07 cm^2
Material	Biocompatible silicone
Bottom: 80496	ibidi Polymer Coverslip of µ-Dish 35 mm high, ibiTreat
Bottom: 80499	No bottom – transfer to your preferred surface

*The gel matrix is not included with this product.



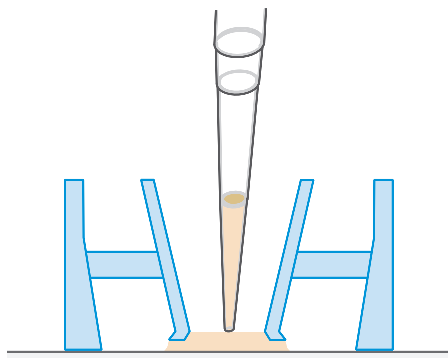
Detailed specifications of the µ-Dish 35 mm, high ibiTreat itself can be found in the instructions on https://ibidi.com/IN_811XX.

We recommend using the micro-Insert 3D for self-insertion (80499) in ibidi µ-Dishes, µ-Slide 1 Well, µ-Slide 2 Well, 6-well plates, 12-well plates or Petri dishes. They can also be used on sterile glass coverslips or glass slides.

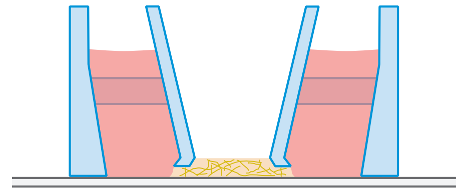
Gel Preparation and Cell Seeding

This section describes the standard protocol, seeding cells on top of a gel matrix. Variations can easily be made by e.g. adding cells to the gel matrix or seeding cells into the outer well. Please see the section Protocol Modifications below for more details on modifying the standard protocol.

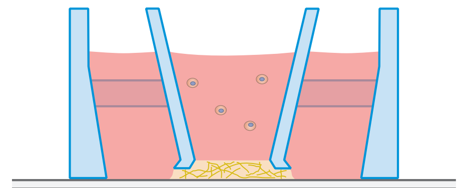
1. Prepare your gel matrix according to the manufacturer's protocol or reference.
2. Fill the inner well with 20 µl liquid gel. Make sure the pipet tip reaches all the way down to the center of the well. Avoid air bubbles and touching the well walls during pipetting.



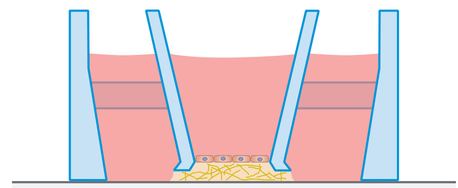
3. Let the gel polymerize as described in the manufacturer's specifications.
4. Visualize and optimize the gel volume for best results. See section Gel Volume Optimization on page 3 for details.
5. Fill the outer well with 250 µl culture medium. Make sure the gel matrix is fully solidified before filling. If 250 µl does not spread well use 2x 125 µl instead, filling from two opposite sides.



6. Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 2–5 × 10^5 cells/ml suspension should result in a 100% optical confluence layer on the matrix after adhesion.
7. Fill the inner well with 25 µl cell suspension. Avoid touching the gel matrix with the pipet tip.



8. Incubate as usual. Control the cell attachment by microscopy.



9. Perform your assay.

NOTE – The above-mentioned volumes for inner and outer well are recommendations. If necessary, change volumes in order to achieve e.g. different liquid levels between inner and outer well.

Protocol Modifications

This section describes modifications from the standard protocol above.

Cells inside the Gel Matrix

To add cells into the gel matrix, use a gel–cell mix with 1–20 × 10^6 cells/ml.

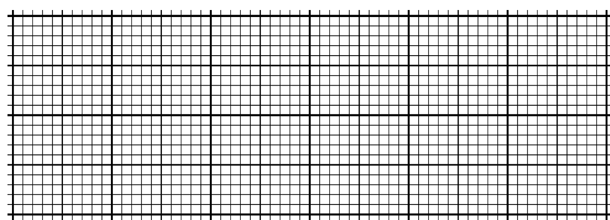
Cells in the Outer Well

To add cells to the outer well, fill it using a cell suspension of 5–11 × 10^4 cells/ml.

Gel Volume Optimization

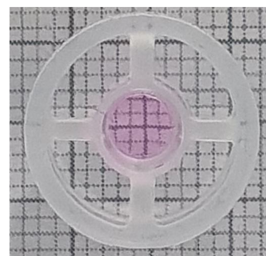
If the inner well contains the correct volume of 20 µl, a flat gel surface is created supporting a homogeneous cell layer and focusing in microscopy. However, because of the gel viscosity, remains in the pipet tip and general pipetting errors the volume ending up in the inner well might be different from the set volume of your pipet.

To visualize the gel flatness accurately millimeter graph paper is a helpful tool:



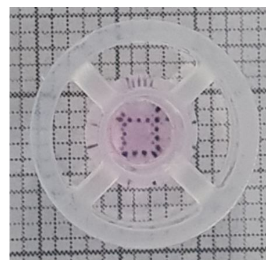
Millimeter graph paper for visualizing the flatness of the gel surface.

After gel filling and gelation, place the insert 1–2 cm above millimeter graph paper. Observe the grid through the gel layer. If a magnification or demagnification effect is visible, change the gel volume in ±1 µl steps.



Optimal gel volume without magnification or demagnification effect.

If the grid of the scale paper appears smaller, the pipetting volume must be increased. If the grid is enlarged, then the pipetting volume must be reduced.



Excess gel volume showing a magnification effect.

Please keep in mind that, depending on the environmental conditions, the volume of a hydrogel can change due to evaporation, swelling or shrinking.

Chemical Compatibility

The following table provides basic information on the chemical and solvent compatibility of the micro-Insert 3D. For a full list of compatible solvents and more information on chemical compatibility, visit ibidi.com/chemicals.

Chemical / Solvent	Compatibility
Methanol	Yes
Ethanol	Yes
Formaldehyde	Yes
Acetone	No
Mineral oil	Yes
Silicone oil	Yes
Immersion oil	See Section “Immersion Oil”

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Immersion Oil

WARNING – When using oil immersion objectives with the ibidi Polymer Coverslip, use only the immersion oils specified in the table below. The use of any non-recommended oil could damage the ibidi Polymer Coverslip. The resulting leakage may harm objectives and microscope components. All immersion oils that are not listed in the table below should be considered as non-compatible.

Company	Product	Ordering No.	Lot Number	Test Date
ibidi	ibidi Immersion Oil 2	50102	24-07-04	07/2024
Cargille	Type A	16482	100592	01/2017
Cargille	Type HF	16245	92192	01/2017
Carl Roth	Immersion oil	X899.1	414220338	01/2017
Leica	Immersion Liquid	11513859	n.a.	03/2023
Leica	Immersion Liquid Type G	11513910	n.a.	04/2024
Nikon	Immersion Oil F2 30cc	MXA22192	n.a.	01/2020
Nikon	Silicone Immersion Oil 30cc	MXA22179	20191101	01/2020
Olympus	Silicone Immersion Oil	SIL300CS-30CC	N4190800	01/2017
Zeiss	Immersol 518 F	444960-0000	220211	03/2023
Zeiss	Immersol 518 F (30 °C)	444970-9010	220816	03/2023
Zeiss	Immersol 518 F (37 °C)	444970-9000	220302	03/2023
Zeiss	Immersol W 2010	444969-0000	101122	04/2012
Zeiss	Immersol Sil 406	444971-9000	80730	03/2023
Zeiss	Immersol G	462959-9901	211117	03/2023

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Further information can be found at ibidi.com. For questions and suggestions, please contact us by e-mail at info@ibidi.com or by telephone at +49 (0)89/520 4617 0.
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