

Coating Protocols for ibidi Labware Products

For optimized cell adhesion protein coatings can be applied to the ibidi labware family. ibidi offers labware with different bottom materials: polymer, glass, Bioinert or ESS (elastically supported surface).

All products with our ibidi Polymer Coverslip bottom in ibiTreat are comparable to standard tissue culture treated labware. This surface permits direct cell growth with a large number of cell lines and primary cells. ibiTreat is a very good support for protein coatings as well. Compared to the ibiTreat surface, the ibidi Polymer Coverslip bottom in the Uncoated version is very hydrophobic. For most adherent cells, a protein coating is required to facilitate cell attachment on the Uncoated surface.

The Bioinert surface cannot be coated with proteins.

1. Recommended Surfaces

Table 1

| Protein Coating | Recommended Surfaces |
|-----------------|--|
| Collagen I | <ul style="list-style-type: none"> • ibiTreat (tissue culture-treated, hydrophilic) • Glass |
| Collagen IV | <ul style="list-style-type: none"> • ibiTreat (tissue culture-treated, hydrophilic) • Uncoated (hydrophobic) • Glass |
| Fibronectin | <ul style="list-style-type: none"> • ibiTreat (tissue culture-treated, hydrophilic) • Uncoated (hydrophobic) • Glass • ESS (elastically supported surface) |
| Poly-L-Lysine | <ul style="list-style-type: none"> • ibiTreat (tissue culture-treated, hydrophilic) • Glass • ESS (elastically supported surface) |
| Poly-D-Lysine | <ul style="list-style-type: none"> • ibiTreat (tissue culture-treated, hydrophilic) • Glass • ESS (elastically supported surface) |

To establish a specific coating or mixture of proteins relevant to a specific research application, we recommend testing the coating procedure on different surfaces in parallel (ibiTreat, Uncoated, and glass). We have observed that some biomolecules adhere differently to hydrophobic and hydrophilic surfaces.

2. Prepare the Coating Solution

All coating solutions are calculated for a specific **amount of protein per area** ($\mu\text{g}/\text{cm}^2$) recommended by the manufacturer's reference.

For Collagen I ($5 \mu\text{g}/\text{cm}^2$):

Dilute the Collagen Type I solution (e.g. ibidi, rat tail, 50202) to the desired concentration using 17.5 mM acetic acid (~0.1% acetic acid).

For Collagen IV: ($1.5 \mu\text{g}/\text{cm}^2$)

Dilute the Collagen Type IV (e.g. Corning, mouse tumor, No. 356233) to the desired concentration using 0.05 M HCl.

For Fibronectin: (1.5 µg/cm²)

Dilute the Fibronectin (e.g. Corning, human plasma, 354008) to the desired concentration using PBS (pH 7.2) without Ca²⁺ and Mg²⁺.

For Poly-L-Lysine: (2 µg/cm²)

Dilute the PLL (e.g. Sigma-Aldrich. 0.01% solution, 100 µg/ml, P4832) to the desired concentration using ultra-pure water.

For Poly-D-Lysine: (5 µg/cm²)

Dilute the PDL (e.g. Corning, No. 354210) to the desired concentration using ultra-pure water.

Use the following volumes [µl] and protein concentrations [µg/ml] in Table 2 and Table 3:

Table 2

| Channel Slides | Volume [µl] | Collagen Type I [µg/ml] | Collagen Type IV [µg/ml] | Fibronectin [µg/ml] | Poly-L-Lysine [µg/ml] | Poly-D-Lysine [µg/ml] |
|----------------------------------|--------------------|-------------------------|--------------------------|---------------------|-----------------------|-----------------------|
| µ-Slide I | 100 | 250 | 75 | 75 | 100 | 250 |
| µ-Slide I 0.2 Luer | 50 | 500 | 150 | 150 | 200 | 500 |
| µ-Slide I 0.4 Luer | 100 | 250 | 75 | 75 | 100 | 250 |
| µ-Slide I 0.6 Luer | 150 | 200 | 60 | 60 | 80 | 200 |
| µ-Slide I 0.8 Luer | 200 | 125 | 38 | 38 | 50 | 125 |
| µ-Slide III 3in1 | 60 | 250 | 75 | 75 | 100 | 250 |
| µ-Slide VI 0.4 | 30 per channel | 250 | 75 | 75 | 100 | 250 |
| µ-Slide VI 0.5 Glass Bottom | 40 per channel | 150 | 45 | 45 | 60 | 150 |
| µ-Slide VI 0.1 | 1.7 per channel | 1000 | 300 | 300 | 400 | 1000 |
| µ-Slide VI - Flat | 30 per channel | 250 | 75 | 75 | 100 | 250 |
| µ-Slide y-shaped | 110 | 250 | 75 | 75 | 100 | 250 |
| µ-Slide Chemotaxis ¹⁾ | 130 per chamber | 130 | 40 | 40 | 55 | 130 |
| µ-Slide Chemotaxis ²⁾ | 6 per chamber | 230 | 70 | 70 | 90 | 230 |
| µ-Slide Membrane ibiPore Flow | 50 (lower channel) | 250 | 75 | 75 | 100 | 250 |
| µ-Slide III 3D Perfusion | 130 per channel | 100 | 30 | 30 | 40 | 100 |
| µ-Slide CorrSight™ Live | 130 per channel | 100 | 30 | 30 | 40 | 100 |

Table 3

| Open Formats | Volume [µl] | Collagen Type I [µg/ml] | Collagen Type IV [µg/ml] | Fibronectin [µg/ml] | Poly-L-Lysine [µg/ml] | Poly-D-Lysine [µg/ml] |
|--------------------------------------|-------------------|-------------------------|--------------------------|---------------------|-----------------------|-----------------------|
| µ-Dish 35 mm, low | 400 | 50 | 15 | 15 | 20 | 50 |
| µ-Dish 35 mm, high ³⁾ | 400 | 50 | 15 | 15 | 20 | 50 |
| µ-Dish 35 mm Quad | 300 | 45 | 13 | 13 | 17 | 45 |
| µ-Dish 35 mm, high ESS ⁴⁾ | 800 | 100 | 30 | 30 | 40 | 100 |
| µ-Dish 50 mm, low | 700 | 60 | 18 | 18 | 25 | 60 |
| Glass Bottom Dish 35 mm | 400 | 50 | 15 | 15 | 20 | 50 |
| µ-Slide 2 Well | 1500 per well | 25 | 8 | 8 | 10 | 25 |
| µ-Slide 4 Well | 700 per well | 30 | 9 | 9 | 12 | 30 |
| µ-Slide 8 Well | 300 per well | 35 | 11 | 11 | 15 | 35 |
| µ-Slide 2 Well Ph+ | 1500 per well | 38 | 11 | 11 | 15 | 38 |
| µ-Slide 4 Well Ph+ | 700 per well | 42 | 12 | 12 | 17 | 42 |
| µ-Slide 2 Well Co-Culture | 70 per minor well | 40 | 12 | 12 | 17 | 40 |
| µ-Slide 18 Well - Flat | 30 per well | 40 | 12 | 12 | 17 | 40 |
| µ-Slide Angiogenesis | 10 per inner well | 125 | 38 | 38 | 50 | 125 |
| µ-Plate 24 Well | 1000 per well | 20 | 6 | 6 | 9 | 20 |
| µ-Plate 96 Well | 300 per well | 35 | 12 | 12 | 15 | 35 |
| µ-Plate 384 Well | 50 per well | 80 | 25 | 25 | 33 | 80 |
| µ-Plate Angiogenesis 96 well | 10 per inner well | 125 | 38 | 38 | 50 | 125 |
| 3 Well Chamber, removable | 1100 per well | 15 | 5 | 5 | 6 | 15 |
| 8 Well Chamber, removable | 400 per well | 35 | 11 | 11 | 15 | 35 |
| 12 Well Chamber, removable | 250 per well | 35 | 11 | 11 | 15 | 35 |

| | | | | | | |
|-----------------------------|--------------|-----|----|----|----|-----|
| Culture-Insert 2 Well | 70 per well | 60 | 18 | 18 | 25 | 60 |
| Culture-Insert 3 Well | 70 per well | 60 | 18 | 18 | 25 | 60 |
| Culture-Insert 4 Well | 110 per well | 60 | 18 | 18 | 25 | 60 |
| micro-Insert 4 Well | 10 per well | 115 | 35 | 35 | 47 | 115 |
| micro-Insert 4 Well FulTrac | 10 per well | 100 | 30 | 30 | 40 | 100 |

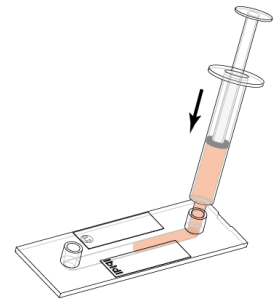
- 1) When coating the entire chamber.
- 2) When coating the observation area only.
- 3) Also valid for glass bottom and Grid-50/Grid-500 versions.
- 4) For the very hydrophobic ESS surface, a higher volume and a higher protein concentration is necessary.

The suggested coating concentration is dependent on the area which is in direct contact with the coating solution. Open wells are coated on the growth area and partially on the side walls. Channels are coated on the entire inner surface. The surface which is coated is called coating area. Please see the APPENDIX for the coating areas which are the basis for the protein concentrations and volumes given above.

3. Fill the Channel or the Well with the Coating Solution Using the Coating Volume from the Tables Above

Work under sterile conditions. Incomplete filling or large air bubbles lead to incomplete coating. The ibiTreat surface is easier to wet completely with the recommended volumes than the hydrophobic, uncoated surface.

Quick dispensing helps filling the channel slides. The very small channels (channel height 0.2 mm and smaller) are filled easier by using a small volume syringe with a male Luer tip as shown on the right.

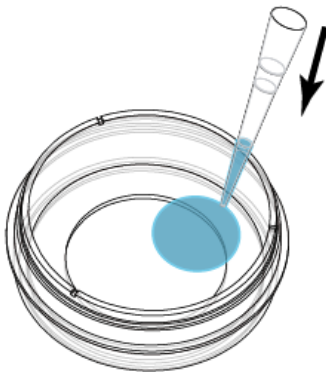


4. Incubate at Room Temperature for 60 Minutes

4a. Washing for Open Wells

Aspirate the well volume completely. Make sure not to touch the coated surface in order to keep the protein coating functional.

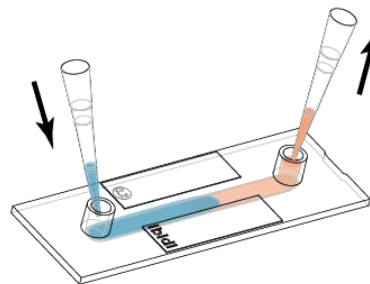
Rinse carefully with ultra-pure water or PBS. For rinsing we recommend using 2 to 3 times the coating volume.



4b. Washing for Channel Slides

Rinse the channel with ultra-pure water or PBS by a continuous liquid exchange making sure that the channel is always filled. For rinsing we recommend using 3 to 5 times the volume of the channel.

When rinsing a channel slide you can easily add solution into one channel end and simultaneously aspirate it on the other side as shown below.



Rinsing thoroughly is necessary to remove all unbound proteins. Any remaining protein may inhibit cell attachment.

- 5. Wells or Channels are ready to use. Optionally, let dry at Room Temperature.**
***Attention: Some coating proteins might degenerate during drying!
Coatings on the ESS surface must not be dried!***

- 6. Store under Sterile Conditions and Use as Soon as Possible**

IMPORTANT NOTE:

Due to the fact that adhesion proteins are biological substances, there can be quality differences between the lots of the manufacturer. Therefore, it is recommended to test every lot number prior to large scale experiments. Prepare and use other coating substrates according to the manufacturer's specifications or reference.

APPENDIX

The concentrations in Table 2 and 3 were calculated based on the following coating areas and volumes:

Table 4

| Channel Slides | Growth Area [cm ²] | Coating Area [cm ²] | Coating Volume [μl] |
|----------------------------------|--------------------------------|---------------------------------|---------------------|
| μ-Slide I | 2.5 | 5.4 | 100 |
| μ-Slide I 0.2 Luer | 2.5 | 5.2 | 50 |
| μ-Slide I 0.4 Luer | 2.5 | 5.4 | 100 |
| μ-Slide I 0.6 Luer | 2.5 | 5.6 | 150 |
| μ-Slide I 0.8 Luer | 2.5 | 5.8 | 200 |
| μ-Slide III 3in1 | 1.23 | 3.05 | 60 |
| μ-Slide VI 0.4 | 0.6 per channel | 1.2 per channel | 30 per channel |
| μ-Slide VI 0.5 Glass Bottom | 0.6 per channel | 1.2 per channel | 40 per channel |
| μ-Slide VI 0.1 | 0.17 per channel | 0.34 per channel | 1.7 per channel |
| μ-Slide VI - Flat | 0.6 per channel | 1.2 per channel | 30 per channel |
| μ-Slide y-shaped | 2.8 | 5.6 | 110 |
| μ-Slide Chemotaxis ¹⁾ | 1.24 per chamber | 3.5 per chamber | 130 per chamber |
| μ-Slide Chemotaxis ²⁾ | 0.06 per chamber | 0.27 per chamber | 6 per chamber |
| μ-Slide Membrane ibiPore Flow | 1.25 (lower channel) | 2.7 (lower channel) | 50 (lower channel) |
| μ-Slide III 3D Perfusion | 0.25 per well | 2.4 per channel | 130 per channel |
| μ-Slide CorrSight™ Live | 0.25 per well | 2.4 per channel | 130 per channel |

Table 5

| Open Formats | Growth Area [cm ²] | Coating Area [cm ²] | Coating Volume [μl] |
|--------------------------------------|--------------------------------|---------------------------------|---------------------|
| μ-Dish 35 mm, low | 3.5 | 4.1 | 400 |
| μ-Dish 35 mm, high ³⁾ | 3.5 | 4.1 | 400 |
| μ-Dish 35 mm Quad | 0.85 per well | 2.46 per well | 300 |
| μ-Dish 35 mm, high ESS ⁴⁾ | 3.5 | 4.1 | 800 |
| μ-Dish 50 mm, low | 7.0 | 7.9 | 700 |
| Glass Bottom Dish 35 mm | 3.14 | 3.7 | 400 |
| μ-Slide 2 Well | 4.8 per well | 7.5 per well | 1500 per well |
| μ-Slide 4 Well | 2.2 per well | 4.1 per well | 700 per well |
| μ-Slide 8 Well | 1.1 per well | 2.2 per well | 300 per well |
| μ-Slide 2 Well Ph+ | 4.8 per well | 11.4 per well | 1500 per well |
| μ-Slide 4 Well Ph+ | 2.2 per well | 5.9 per well | 700 per well |
| μ-Slide 2 Well Co-Culture | 0.4 per minor well | 0.55 per minor well | 70 per minor well |
| μ-Slide 18 Well - Flat | 0.2 per well | 0.25 per well | 30 per well |
| μ-Slide Angiogenesis | 0.12 per well | 0.23 per well | 10 per inner well |
| μ-Plate 24 Well Black | 1.9 per well | 4.3 per well | 1000 per well |
| μ-Plate 96 Well Black | 0.55 per well | 2.35 per well | 300 per well |
| μ-Plate 384 Well Clear | 0.11 per well | 0.80 per well | 50 per well |
| μ-Plate Angiogenesis 96 Well | 0.12 per well | 0.23 per well | 10 per inner well |
| 3 Well Chamber, removable | 1.66 per well | 3.37 per well | 1100 per well |
| 8 Well Chamber, removable | 0.93 per well | 2.63 per well | 400 per well |
| 12 Well Chamber, removable | 0.56 per well | 1.9 per well | 250 per well |
| Culture-Insert 2 Well | 0.22 per well | 0.82 per well | 70 per well |
| Culture-Insert 3 Well | 0.22 per well | 0.82 per well | 70 per well |
| Culture-Insert 4 Well | 0.35 per well | 1.23 per well | 110 per well |
| micro-Insert 4 Well | 0.03 per well | 0.23 per well | 10 per well |
| micro-Insert 4 Well FulTrac | 0.0012 per well | 0.188 per well | 10 per well |

¹⁾ When coating the entire chamber.

²⁾ When coating the observation area only.

³⁾ Also valid for glass bottom and Grid-50/Grid-500 versions.

⁴⁾ For the very hydrophobic ESS surface, a higher volume is necessary to cover the surface.