

## Coating Protocols for ibidi Labware

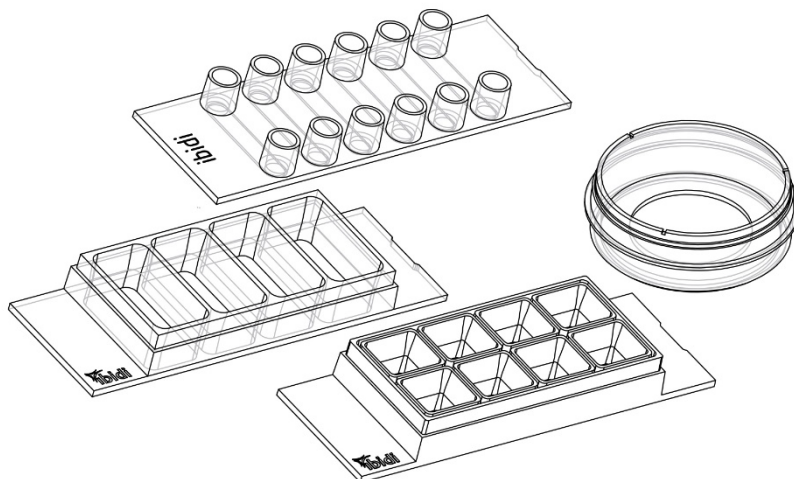
The growth, development, and signaling of cultured cells strongly depends on the used surface. The ibidi labware has a thin coverslip bottom ([ibidi Polymer Coverslip](#) or [Glass Coverslip](#), both with excellent optical quality). For optimized cell adhesion, protein coatings can be applied to the ibidi labware family.

This Application Note provides a detailed protocol on how to coat the ibidi labware, as well as important information about the required volumes and protein concentrations.

ibidi also offers a high-quality [Collagen Type I, Rat Tail](#) and [Collagen Type I, Bovine](#) solutions for preparing 3D gels and surface coatings.

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## 1 Recommended Surfaces for Different Coatings

The ibidi labware is available with several surfaces:

- **ibiTreat (tissue culture-treated) surface on the ibidi Polymer Coverslip:** Excellent adhesion of adherent cells, hydrophilic surface; coating possible, but not needed for most adherent cell types
- **Hydrophobic, Uncoated surface on the ibidi Polymer Coverslip:** Weak adhesion of adherent cells, hydrophobic surface; coating usually required for cell adhesion
- **Bioinert surface on the ibidi Polymer Coverslip:** No adhesion of adherent cells or any protein, stable long-term passivation; no coating possible
- **ESS (elastically supported surface) on the ibidi Polymer Coverslip:** Culture of adherent cells on an elastic, very hydrophobic surface; coating required for cell adhesion
- **Glass surface on the ibidi Glass Coverslip:** Adhesion of adherent cells; coating might be required for optimal cell adhesion

Protein Coating	Recommended Surfaces
Collagen I	ibiTreat Glass
Collagen IV	ibiTreat Hydrophobic, Uncoated Glass
Fibronectin	ibiTreat Hydrophobic, Uncoated ESS Glass
Laminin	ibiTreat Hydrophobic, Uncoated
Poly-L-Lysine	ibiTreat ESS Glass
Poly-D-Lysine	ibiTreat ESS Glass

Some biomolecules adhere differently to hydrophobic and hydrophilic surfaces (e.g., ibiTreat or glass). Therefore, we recommend testing a specific coating or a protein mixture on different surfaces before using them in an experiment.

Free samples of the ibidi labware can be ordered at: [ibidi.com/freesample](https://www.ibidi.com/freesample)

## 2 Recommended Protein Concentrations and Coating Volumes for ibidi Labware

Here you find precalculated values of some standard coatings for the different ibidi labware products. There is an equation for calculating the protein concentration in the Appendix.

In this Application Note, we used the following coating solutions:

### **Collagen I** (ibidi, Rat Tail, 50202 or ibidi, Bovine, 50302)

Recommended protein amount per area: 5 µg/cm<sup>2</sup>. Dilute the Collagen Type I to the required concentration using a) for rat tail: 17.5 mM acetic acid (~0.1% acetic acid) or b) for bovine: 0.1 M acetic acid (~0.6% acetic acid).

### **Collagen IV** (Corning, mouse tumor, No. 356233)

Recommended protein amount per area: 1.5 µg/cm<sup>2</sup>. Dilute the Collagen Type IV to the required concentration using 0.05 M HCl.

### **Fibronectin** (Corning, human plasma, 354008)

Recommended protein amount per area: 1.5 µg/cm<sup>2</sup>. Dilute the Fibronectin to the required concentration using PBS (pH 7.2) without Ca<sup>2+</sup> and Mg<sup>2+</sup>.

### **Laminin** (BioLamina, human recombinant laminin, Biolaminin® 521, LN521)

Recommended protein amount per area: 0.5 µg/cm<sup>2</sup>. Dilute the Laminin to the required concentration using PBS (pH 7.2) without Ca<sup>2+</sup> and Mg<sup>2+</sup>.

### **Poly-L-Lysine** (Sigma-Aldrich, 0.01% solution, 100 µg/ml, P4832)

Recommended protein amount per area: 2 µg/cm<sup>2</sup>. Dilute the PLL to the required concentration using ultra-pure water.

### **Poly-D-Lysine** (Corning, No. 354210)

Recommended protein amount per area: 5 µg/cm<sup>2</sup>. Dilute the PDL to the required concentration using ultra-pure water.

If any coating protein products other than those specified here are being used, please follow the manufacturer's instructions for the recommended protein amount per area and preparation.

**IMPORTANT NOTE:** Since adhesion proteins are biological substances, there can be quality differences between the lots of a manufacturer. Therefore, we recommend testing every lot number, especially prior to large scale experiments.

## Recommended Coating Volumes and Protein Concentrations for ibidi Labware

	Protein Concentration						
	Coating Volume [µl]	Collagen I [µg/ml]	Collagen IV [µg/ml]	Fibronectin [µg/ml]	Laminin [µg/ml]	Poly-L-Lysine [µg/ml]	Poly-D-Lysine [µg/ml]
µ-Dish 35 mm, low <sup>1)</sup>	400	50	15	15	5	20	50
µ-Dish 35 mm, high <sup>1)</sup>	400	50	15	15	5	20	50
µ-Dish 35 mm, high ESS <sup>2)</sup>	800	100	30	30	10	40	100
µ-Dish 35 mm Quad	300	45	13	13	4.5	17	45
Glass Bottom Dish 35 mm	400	50	15	15	5	20	50

<sup>1)</sup> Also valid for glass bottom and Grid-50/Grid-500 versions.

<sup>2)</sup> For the very hydrophobic ESS surface, a higher volume and a higher protein concentration are necessary

	Protein Concentration						
	Coating Volume [μl]	Collagen I [μg/ml]	Collagen IV [μg/ml]	Fibronectin [μg/ml]	Laminin [μg/ml]	Poly-L-Lysine [μg/ml]	Poly-D-Lysine [μg/ml]
μ-Slide 1 Well <sup>3)</sup>	3000	24	7	7	2.4	10	24
μ-Slide 2 Well <sup>3)</sup>	1500 / well	25	8	8	2.5	10	25
μ-Slide 2 Well Ph+ <sup>3)</sup>	1500 / well	38	11	11	3.8	15	38
μ-Slide 2 Well Co-Culture	70 / minor well	40	12	12	4.0	17	40
μ-Slide 4 Well <sup>3)</sup>	700 / well	30	9	9	3.0	12	30
μ-Slide 4 Well Ph+ <sup>3)</sup>	700 / well	42	12	12	4.2	17	42
μ-Slide 8 Well <sup>3)</sup>	300 / well	35	11	11	3.5	15	35
μ-Slide 8 Well high <sup>3)</sup>	300 / well	35	11	11	3.5	15	35
μ-Slide 15 Well 3D <sup>3)</sup>	10 / inner well	125	38	38	12.5	50	125
μ-Slide 18 Well <sup>3)</sup>	100 / well	58	17	17	5.8	23	58
μ-Slide 18 Well - Flat	30 / well	40	12	12	4.0	17	40
μ-Slide I	100	250	75	75	25	100	250
μ-Slide I 0.2 Luer	50	500	150	150	50	200	500
μ-Slide I 0.4 Luer	100	250	75	75	25	100	250
μ-Slide I 0.6 Luer	150	200	60	60	20	80	200
μ-Slide I 0.8 Luer	200	145	44	44	14.5	60	145
μ-Slide I 0.2 Luer Glass Bottom	62.5	420	125	125	42	170	420
μ-Slide I 0.4 Luer Glass Bottom	112.5	250	75	75	25	100	250
μ-Slide I 0.6 Luer Glass Bottom	162.5	175	52	52	17.5	70	175
μ-Slide I 0.8 Luer Glass Bottom	212.5	135	41	41	13.5	55	135
μ-Slide I Luer 3D	16 / well	100	30	30	10	40	100
μ-Slide III 3in1	60	250	75	75	25	100	250
μ-Slide VI 0.4	30 / channel	250	75	75	25	100	250
μ-Slide VI 0.5 Glass Bottom	40 / channel	150	45	45	15	60	150
μ-Slide VI 0.1	1.7 / channel	1000	300	300	100	400	1000
μ-Slide VI - Flat	30 / channel	250	75	75	25	100	250
μ-Slide y-shaped	110	250	75	75	25	100	250
μ-Slide Chemotaxis <sup>4)</sup>	130 / chamber	130	40	40	13	55	130
μ-Slide Chemotaxis <sup>5)</sup>	6 / chamber	230	70	70	23	90	230
μ-Slide ibiPore SiN	50 (lower channel)	250	75	75	25	100	250
μ-Slide III 3D Perfusion	130 / channel	100	30	30	10	40	100
μ-Slide Spheroid Perfusion	3.5 / well	140	40	40	14	55	140
μ-Plate 6 Well	5000 / well	15	4	4	1.5	6	15
μ-Plate 24 Well	1000 / well	20	6	6	2	9	20
μ-Plate 96 Well 3D	10 / inner well	125	38	38	12.5	50	125
μ-Plate 96 Well Round <sup>3)</sup>	200 / well	40	12	12	4	16	40
μ-Plate 96 Well Square <sup>3)</sup>	300 / well	35	12	12	3.5	15	35
μ-Plate 384 Well Glass Bottom	50 / well	80	25	25	8	33	80
3 Well Chamber, removable	1100 / well	15	5	5	1.5	6	15
8 Well Chamber, removable	400 / well	35	11	11	3.5	15	35
12 Well Chamber, removable	250 / well	35	11	11	3.5	15	35
Culture-Insert 2 Well	70 / well	60	18	18	6	25	60
Culture-Insert 3 Well	70 / well	60	18	18	6	25	60

	Protein Concentration						
	Coating Volume [μl]	Collagen I [μg/ml]	Collagen IV [μg/ml]	Fibronectin [μg/ml]	Laminin [μg/ml]	Poly-L-Lysine [μg/ml]	Poly-D-Lysine [μg/ml]
Culture-Insert 4 Well	110 / well	60	18	18	6	25	60
micro-Insert 4 Well	10 / well	115	35	35	11.5	47	115
micro-Insert 4 Well FulTrac	10 / well	100	30	30	10	40	100

<sup>3)</sup> Also valid for the glass bottom version.

<sup>4)</sup> When coating the entire chamber (observation area + 2 reservoirs).

<sup>5)</sup> When coating the observation area only.

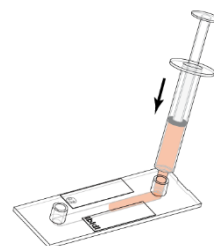
### 3 Coating Protocol

All steps need to be performed under sterile conditions.

1. Check the required protein concentration and coating volume, then dilute the coating solution to the desired concentration (see Section 2).
2. Fill the vessel (e.g., a channel or a well) with the coating solution, using the coating volume in Section 2.

Incomplete filling or large air bubbles will lead to incomplete coating. The ibiTreat surface is easier to wet completely with the recommended volumes than the Hydrophobic, Uncoated surface.

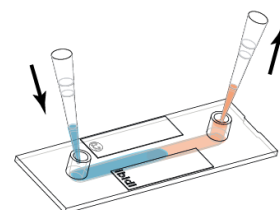
Quick dispensing helps when filling the channel slides. Very small channels (channel height 0.2 mm and below) can be filled easier by using a small volume syringe with a male Luer tip, as shown on the right.



3. Incubate at room temperature for 60 minutes.
4. Wash thoroughly.

**Attention:** It is necessary to remove all unbound proteins. Any remaining protein may inhibit cell attachment.

- a. Open wells: Aspirate the well volume completely. Make sure not to touch the coated surface to keep the protein coating functional. Rinse carefully with ultra-pure water or PBS. For rinsing, we recommend using 2–3x the coating volume.
- b. Channels: Rinse the channel with ultra-pure water or PBS by a continuous liquid exchange using 3–5x the channel volume. Make sure that the channel is always filled by adding the washing solution into one channel end, followed by aspiration from the opposite end, as shown on the right.



5. Optionally, let dry at room temperature.

**Attention:** Some coating proteins might degenerate during drying! Coatings on the ESS surface **must not** be dried!

6. The labware is now coated and ready-to-use. Introduce cells and medium immediately or store it under sterile conditions and use as soon as possible.

## 4 Appendix

### 4.1 Preparation of the Coating Solution: Calculating the Necessary Concentration for ibidi Labware

Before preparing a coating, you need to calculate the protein concentration and the coating volume required for the chosen labware. This Application Note contains the precalculated values of some standard coatings for the different ibidi labware products.

All calculations are based on the amount of **protein per area (P)** [ $\mu\text{g}/\text{cm}^2$ ], which is specific for the coating protein being used, and is recommended by the manufacturer. Importantly, optimal conditions for cell attachment can vary and are influenced by the cell type and application. The typical range for the protein amount per area is 1–5  $\mu\text{g}/\text{cm}^2$ .

The calculated **protein concentration (c)** [ $\mu\text{g}/\text{ml}$ ] depends on the **coating area (A)** [ $\text{cm}^2$ ] and the **coating volume (V)** [ $\mu\text{l}$ ] that is needed to cover the coating area.

The coating area, which is in direct contact with the coating solution, is defined by the used labware: Channels are coated on the entire inner surface; open wells are coated on the growth area and partially on the side walls. Please see Section 4.2 for the coating areas, which are the basis for the protein concentrations and volumes calculated in this section.

The required protein concentration for the coating of different labware is calculated as follows:

$$c [\mu\text{g}/\text{ml}] = \frac{P [\mu\text{g}/\text{cm}^2] \times A [\text{cm}^2] \times 1000}{V [\mu\text{l}]}$$

### 4.2 Coating Areas and Coating Volumes of the ibidi Labware

The protein concentrations for the ibidi labware in Section 2 were calculated based on the following coating areas and coating volumes. The growth area is listed for information purposes only.

	Growth Area [ $\text{cm}^2$ ]	Coating Area [ $\text{cm}^2$ ]	Coating Volume [ $\mu\text{l}$ ]
$\mu$ -Dish 35 mm, low <sup>1)</sup>	3.5	4.1	400
$\mu$ -Dish 35 mm, high <sup>1)</sup>	3.5	4.1	400
$\mu$ -Dish 35 mm, high ESS <sup>2)</sup>	3.5	4.1	800
$\mu$ -Dish 35 mm Quad	0.85 / well	2.46 / well	300
Glass Bottom Dish 35 mm	3.14	3.7	400
$\mu$ -Slide 1 Well <sup>3)</sup>	10.6	14.6	3000
$\mu$ -Slide 2 Well <sup>3)</sup>	4.8 / well	7.5 / well	1500 / well
$\mu$ -Slide 2 Well Ph+ <sup>3)</sup>	4.8 / well	11.4 / well	1500 / well
$\mu$ -Slide 2 Well Co-Culture	0.4 / minor well	0.55 / minor well	70 / minor well
$\mu$ -Slide 4 Well <sup>3)</sup>	2.2 / well	4.1 / well	700 / well
$\mu$ -Slide 4 Well Ph+ <sup>3)</sup>	2.2 / well	5.9 / well	700 / well
$\mu$ -Slide 8 Well <sup>3)</sup>	1.0 / well	2.2 / well	300 / well
$\mu$ -Slide 8 Well high <sup>3)</sup>	1.0 / well	2.2 / well	300 / well
$\mu$ -Slide 15 Well 3D <sup>3)</sup>	0.12 / well	0.23 / well	10 / inner well
$\mu$ -Slide 18 Well <sup>3)</sup>	0.34 / well	1.15 / well	100 / well
$\mu$ -Slide 18 Well - Flat	0.2 / well	0.25 / well	30 / well

<sup>1)</sup> Also valid for glass bottom and Grid-50/Grid-500 versions.

<sup>2)</sup> For the very hydrophobic ESS surface, a higher volume and a higher protein concentration are necessary.

<sup>3)</sup> Also valid for the glass bottom version.

	Growth Area [cm <sup>2</sup> ]	Coating Area [cm <sup>2</sup> ]	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 I 0.2 Luer Glass Bottom	2.5	5.2	62.5
μ-Slide I 0.4 Luer Glass Bottom	2.5	5.4	112.5
μ-Slide I 0.6 Luer Glass Bottom	2.5	5.6	162.5
μ-Slide I 0.8 Luer Glass Bottom	2.5	5.8	212.5
μ-Slide I Luer 3D	0.21 / well	0.34 / well	16 / well
μ-Slide III 3in1	1.23	3.05	60
μ-Slide VI 0.4	0.6 / channel	1.2 / channel	30 / channel
μ-Slide VI 0.5 Glass Bottom	0.6 / channel	1.2 / channel	40 / channel
μ-Slide VI 0.1	0.17 / channel	0.34 / channel	1.7 / channel
μ-Slide VI - Flat	0.6 / channel	1.2 / channel	30 / channel
μ-Slide y-shaped	2.8	5.6	110
μ-Slide Chemotaxis <sup>4)</sup>	1.24 / chamber	3.5 / chamber	130 / chamber
μ-Slide Chemotaxis <sup>5)</sup>	0.06 / chamber	0.27 / chamber	6 / chamber
μ-Slide ibiPore SiN	1.25 (lower channel)	2.7 (lower channel)	50 (lower channel)
μ-Slide III 3D Perfusion	0.25 / well	2.4 / channel	130 / channel
μ-Slide Spheroid Perfusion	0.005 / well	0.097 / well	3.5 / well
μ-Plate 6 Well	9.1 / well	14.9 / well	5000 / well
μ-Plate 24 Well	1.54 / well	4.4 / well	1000 / well
μ-Plate 96 Well 3D	0.12 / well	0.23 / well	10 / inner well
μ-Plate 96 Well Round 3)	0.3 / well	1.61 / well	200 / well
μ-Plate 96 Well Square 3)	0.55 / well	2.35 / well	300 / well
μ-Plate 384 Well Glass Bottom	0.11 / well	0.80 / well	50 / well
3 Well Chamber, removable	1.66 / well	3.37 / well	1100 / well
8 Well Chamber, removable	0.93 / well	2.63 / well	400 / well
12 Well Chamber, removable	0.56 / well	1.9 / well	250 / well
Culture-Insert 2 Well	0.22 / well	0.82 / well	70 / well
Culture-Insert 3 Well	0.22 / well	0.82 / well	70 / well
Culture-Insert 4 Well	0.35 / well	1.23 / well	110 / well
micro-Insert 4 Well	0.03 / well	0.23 / well	10 / well
micro-Insert 4 Well FulTrac	0.0012 / well	0.188 / well	10 / well

<sup>3)</sup> Also valid for the glass bottom version.

<sup>4)</sup> When coating the entire chamber (observation area + 2 reservoirs).

<sup>5)</sup> When coating the observation area only.