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	• ibiTreat (tissue culture-treated, hydrophilic) • Glass
Collagen IV	• ibiTreat (tissue culture-treated, hydrophilic) • Uncoated (hydrophobic) • Glass
Fibronectin	• ibiTreat (tissue culture-treated, hydrophilic) • Uncoated (hydrophobic) • Glass • ESS (elastically supported surface)
Poly-L-Lysine	• ibiTreat (tissue culture-treated, hydrophilic) • Glass • ESS (elastically supported surface)
Poly-D-Lysine	• 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** (µg/cm²) recommended by the manufacturer's reference.

For Collagen I (5 µg/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 µg/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/cm^2)
Dilute the Fibronectin (e.g. Corning, human plasma, 354008) to the desired concentration using PBS (pH 7.2) without Ca^{2+} and Mg^{2+}.

For Poly-L-Lysine: (2 µg/cm^2)
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/cm^2)
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

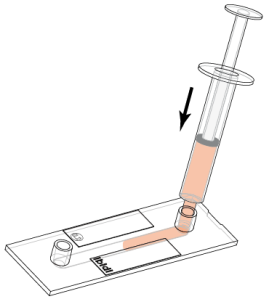
[1] When coating the entire chamber.
[2] When coating the observation area only.
[3] Also valid for glass bottom and Grid-50/Grid-500 versions.
[4] For the very hydrophobic ESS surface, a higher volume and a higher protein concentration is necessary.

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.

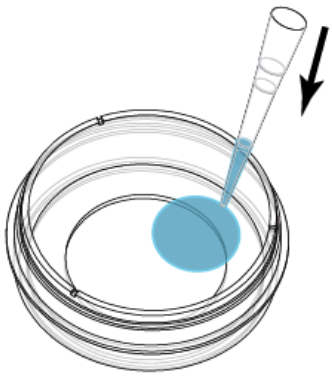


4. Incubate at Room Temperature for 60 Minutes

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.

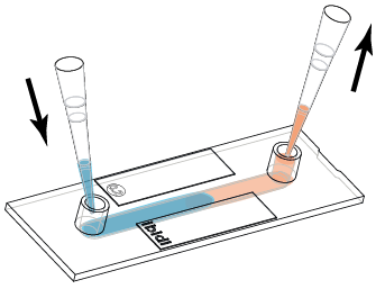
Rinse carefully with ultra-pure water or PBS. For rinsing we recommend using 2 to 3 times the coating volume.



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 [cm²]	Coating Area [cm²]	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 [cm²]	Coating Area [cm²]	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.