Coating procedures for ibidi µ-Slides and µ-Dishes

For optimized cell adhesion there are different treatments and coatings for the ibidi labware family. The ibiTreat surface is comparable to standard tissue culture treated labware. This surface permits direct cell growth with a large number of cell lines and primary cells. Compared to the ibiTreat surface, the uncoated surface is very hydrophobic. The uncoated surface must be coated with a protein solution for the adhesion of most cells.

1. Recommended Surfaces

For Collagen I: ibiTreat (tissue culture treated)
For Collagen IV: ibiTreat (tissue culture treated) or hydrophobic, uncoated
For Fibronectin: ibiTreat (tissue culture treated) or hydrophobic, uncoated
For Poly-L-Lysine: ibiTreat (tissue culture treated)
For Poly-D-Lysine: ibiTreat (tissue culture treated)

To establish a specific coating relevant to a specific research application, we recommend testing the coating procedure on both uncoated and ibiTreat µ-Slides. We have observed that some biomolecules adhere differently to hydrophobic and hydrophilic plastic surfaces.

Please note that there is no uncoated version of the µ-Slide Chemotaxis. Use the ibiTreat surface for all coatings.

Some products are also offered with a glass bottom. Glass can also be coated according to the specifications below.

The ESS surface needs higher protein concentrations for an effective coating and must not be dried after coating.

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 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 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 protein concentrations [µg/ml]:

Channel Slides

	Collagen I	Collagen IV	Fibronectin	Poly-L-Lysine	Poly-D-Lysine
µ-Slide I	250	75	75	100	250
µ-Slide I 0.2 Luer	500	150	150	200	500
µ-Slide I 0.4 Luer	250	75	75	100	250
µ-Slide I 0.6 Luer	200	60	60	80	200
µ-Slide I 0.8 Luer	125	38	38	50	125
µ-Slide III 3in1	250	75	75	100	250
µ-Slide VI 0.4	250	75	75	100	250
µ-Slide VI 0.1	1000	300	300	400	1000
µ-Slide VI - Flat	250	75	75	100	250
µ-Slide y-shaped	250	75	75	100	250
µ-Slide Chemotaxis [1)]	130	40	40	55	130
µ-Slide Chemotaxis [2)]	230	70	70	90	230
µ-Slide Chemotaxis 2D [1)]	150	45	45	60	150
µ-Slide Chemotaxis 2D [2)]	330	100	100	133	330
µ-Slide Membrane ibiPore Flow	250	75	75	100	250
µ-Slide III 3D Perfusion	100	30	30	40	100
µ-Slide CorrSight™ Live	100	30	30	40	100

Open Formats

	Collagen I	Collagen IV	Fibronectin	Poly-L-Lysine	Poly-D-Lysine
µ-Dish 35 mm, low	50	15	15	20	50
µ-Dish 35 mm, high	50	15	15	20	50
µ-Dish 35 mm, high ESS [3)]	100	30	30	40	100
µ-Dish 50 mm, low	60	18	18	25	60
µ-Slide 2 well	25	8	8	10	25
µ-Slide 4 well	30	9	9	12	30
µ-Slide 8 well	35	11	11	15	35
µ-Slide 2 well Ph+	38	11	11	15	38
µ-Slide 4 well Ph+	42	12	12	17	42
µ-Slide 2 Well Co-Culture	40	12	12	17	40
µ-Slide 18 well - Flat	40	12	12	17	40
µ-Slide Angiogenesis	125	38	38	50	125
µ-Plate 24 well	20	6	6	9	20
µ-Plate 96 well	35	12	12	15	35
µ-Plate Angiogenesis 96 well	125	38	38	50	125
3 Well Chamber, removable	15	5	5	6	15
8 Well Chamber, removable	35	11	11	15	35
12 Well Chamber, removable	35	11	11	15	35
Culture-Insert 2 Well	60	18	18	25	60
Culture-Insert 3 Well	60	18	18	25	60
Culture-Insert 4 Well	60	18	18	25	60
micro-Insert 4 Well	115	35	35	47	115
micro-Insert 4 Well FulTrac	100	30	30	40	100

[1)] When coating the full chamber.
[2)] When coating the observation area only.
[3)] For the very hydrophobic ESS surface, a higher protein concentration is necessary.

Keep in mind that all walls inside the channels are coated in the ibidi channel slides. Open formats are coated not only on the growth area but also partially on the side walls. The coating areas are valid for the exact coating volumes in the table only.

The dilutions above were calculated using the following coating areas and volumes. The coating area is the area which is in contact with the liquid, thus coated.

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 0.1	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 Chemotaxis 2D [1)]	0.96 per chamber	2.4 per chamber	80 per chamber
µ-Slide Chemotaxis 2D [2)]	0.07 per chamber	0.39 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

Open Formats

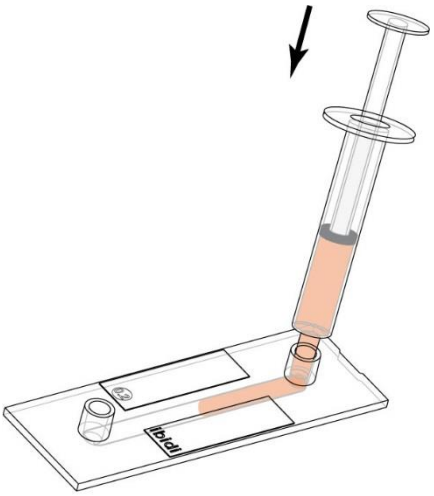
	Growth Area [cm²]	Coating Area [cm²]	Coating Volume [µl]
µ-Dish 35 mm, low	3.5	4.1	400
µ-Dish 35 mm, high	3.5	4.1	400
µ-Dish 35 mm, high ESS [3)]	3.5	4.1	800
µ-Dish 50 mm, low	7.0	7.9	700
µ-Slide 2 well	4.8 per well	7.5 per well	1500 per well
µ-Slide 4 well	2.2 per well	4.1 per well	700 per well
µ-Slide 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	1.9 per well	4.3 per well	1000 per well
µ-Plate 96 well	0.55 per well	2.35 per well	300 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 full chamber.
[2)] When coating observation area only.
[3)] Also valid for glass bottom and Grid-50/Grid-500 versions.

Keep in mind all walls inside the channels are coated in the ibidi channel slides. Open formats are coated not only on the growth area but also partially on the side walls. The coating areas are valid for the exact coating volumes in the table only.

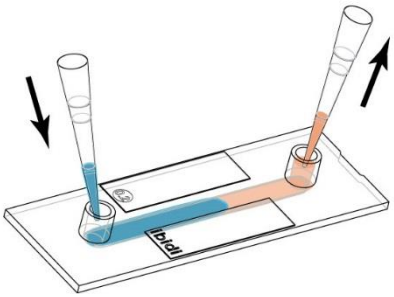
3. **Fill the channel or the well with the coating solution using the coating volume from the tables above.**

Quick dispensing helps fill the channel slides more easily. Work under sterile conditions. Incomplete filling, or large air bubbles, leads to reduced coating. The ibiTreat surface is easier to completely wet with the recommended volumes than the hydrophobic, uncoated surface.
The very small channels (channel height 0.2 mm and smaller) are filled more easily by using a small volume syringe with a male Luer tip as shown on the right.



4. **Incubate at room temperature for 60 minutes.**
5. **Aspirate the channel or the well volume completely.**
6. **Rinse carefully with ultra-pure water or PBS.**

For rinsing we recommend using 5-10 times the volume of the channel or well. When rinsing a channel slide you can easily add solution into one channel end and simultaneously aspirate it on the other side as shown.



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

7. **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!**
8. **Store under sterile conditions and use as soon as possible.**

IMPORTANT NOTES:
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.