Coating procedures for ibidi µ-Slides and µ-Dishes

For optimized cell adhesion there are different treatments and coatings for the µ-Slide family. The ibiTreat surface is comparable with standard tissue culture treated plastic ware. This surface permits direct cell growth as shown with a large number of cell lines and primary cells. Compared to ibiTreat, uncoated has a very hydrophobic surface, which must be coated with adhesion factors for the adhesion of most cells.

1. Recommended surfaces

For Collagen I: ibiTreat (tissue culture treated) or hydrophobic, uncoated
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)

If you want to do a different coating by yourself, we recommend trying both surfaces, ibiTreat and uncoated. Some products are also offered with glass bottom.

Please note that there is no uncoated version of the µ-Slides Chemotaxis 2D and 3D due to appropriate filling. In this case, use the ibiTreat surface for all coatings.

2. Prepare the coating solution

All coating solutions are calculated for a certain **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 2 mM acetic acid (~0.1% acetic acid).

For Collagen IV: (1.5 µg/cm^2)
Dilute the Collagen IV (e.g. Becton-Dickinson, 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. Becton-Dickinson, 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. Becton-Dickinson, No. 35 4210) 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 0.1 Luer	1000	300	300	400	1000
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 0.1	1000	300	300	400	1000
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 upright 0.8	200	60	60	80	200
µ-Slide Chemotaxis 2D [1)]	150	45	45	60	150
µ-Slide Chemotaxis 2D [2)]	330	100	100	133	330
µ-Slide Chemotaxis 3D [1)]	130	40	40	55	130
µ-Slide Chemotaxis 3D [2)]	230	70	70	90	230

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 [3)]	50	15	15	20	50
µ-Dish 50 mm, low	60	18	18	25	60
µ-Slide 8 well	35	11	11	15	35
µ-Slide 2x9 well	40	12	12	17	40
µ-Slide 18 well	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 384 well	70	24	24	30	70
µ-Plate Angiogenesis 96 well	125	38	38	50	125
µ-Chamber 12 well	35	11	11	15	35
Culture-Insert	60	18	18	25	60
micro-Insert 4 well	115	35	35	47	115

[1)] When coating full chamber
[2)] When coating observation area only
[3)] Also valid for glass bottom version and ESS (elastically supported surface) version.

Keep in mind that channel slides are coated on all walls inside the channel. 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	100.0
µ-Slide I 0.1 Luer	2.5	5.1	25.0
µ-Slide I 0.2 Luer	2.5	5.2	50.0
µ-Slide I 0.4 Luer	2.5	5.4	100.0
µ-Slide I 0.6 Luer	2.5	5.6	150.0
µ-Slide I 0.8 Luer	2.5	5.8	200.0
µ-Slide III 0.1	0.43	0.86	4.5 per channel
µ-Slide III 3in1	1.23	3.05	60.0
µ-Slide VI 0.4	0.60 per channel	1.20 per channel	30.0 per channel
µ-Slide VI 0.1	0.17 per channel	0.34 per channel	1.7 per channel
µ-Slide VI flat	0.60 per channel	1.20 per channel	30.0 per channel
µ-Slide y-shaped	2.8	5.6	110.0
µ-Slide upright 0.8	4.35	10.31	250.0
µ-Slide Chemotaxis 2D 1)	0.96 per chamber	2.40 per chamber	80.0 per chamber
µ-Slide Chemotaxis 2D 2)	0.07 per chamber	0.39 per chamber	6.0 per chamber
µ-Slide Chemotaxis 3D 1)	1.24 per chamber	3.50 per chamber	130.0 per chamber
µ-Slide Chemotaxis 3D 2)	0.06 per chamber	0.27 per chamber	6.0 per chamber

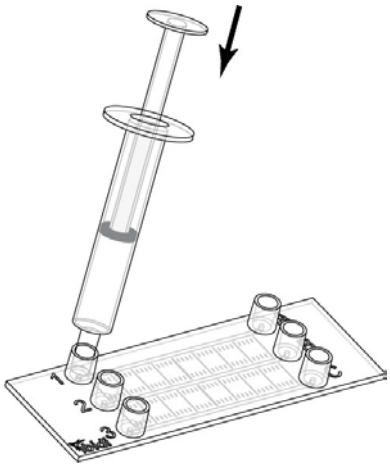
Open Formats

	Growth Area [cm²]	Coating Area [cm²]	Coating Volume [µl]
µ-Dish 35mm, low	3.5	4.1	400
µ-Dish 35mm, high 3)	3.5	4.1	400
µ-Dish 50mm, low	7.0	7.9	700
µ-Slide 8 well	1.10 per well	2.20 per well	300 per well
µ-Slide 2x9 well	0.40 per minor well	0.55 per minor well	70 per minor well
µ-Slide 18 well	0.20 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	1.90 per well	4.30 per well	1000 per well
µ-Plate 96 well	0.55 per well	2.35 per well	300 per well
µ-Plate 384 well	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
µ-Chamber 12 well	0.56 per well	1.90 per well	250 per well
Culture-Insert	0.22 per well	0.82 per well	70 per well
micro-Insert 4 well	0.03 per well	0.23 per well	10 per well

1) When coating full chamber.
2) When coating observation area only.
3) Also valid for glass bottom version and ESS (elastically supported surface) version.

Keep in mind that channel slides are coated on all walls inside the channel. 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.

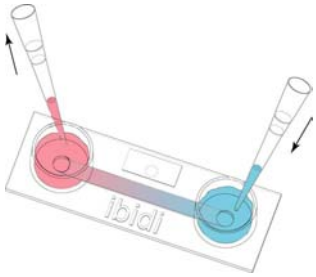
3. **Fill the channel or the well with the coating solution using the coating volume from the tables above.**



Quick dispensing helps to fill the channel slides easier. Work under sterile conditions. Consider that incomplete filling or large air bubbles lead to reduced coating. Due to hydrophilicity, the ibiTreat surface gets wetted much better than the hydrophobic, uncoated surface.
The very small channels (channel height smaller 0.2 mm) 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 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 non-bound proteins. If not removed, those remains 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!**
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 performing tests with every lot number. Prepare and use other coating substrates according to the manufacturer's specifications or reference.