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 hydrophobic surface, which must be coated with adhesion factors for the adhesion of most cells.

1. Recommended surfaces

For Collagen IV: hydrophobic, uncoated

For Fibronectin: 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 ibiTreat version of µ-Slide V, µ-Slide I $^{0.1}$ Luer, µ-Slide III $^{0.1}$, and µ-Plate 384 well. In those cases, use the hydrophobic, uncoated 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 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 IV	Fibronectin	Poly-L-Lysine	Poly-D-Lysine
µ-Slide I	75	75	100	250
µ-Slide I $^{0.1}$ Luer	300	300	400	1000
µ-Slide I $^{0.2}$ Luer	150	150	200	500
µ-Slide I $^{0.4}$ Luer	75	75	100	250
µ-Slide I $^{0.6}$ Luer	60	60	80	200
µ-Slide I $^{0.8}$ Luer	38	38	50	125
µ-Slide III $^{0.1}$	300	300	400	1000
µ-Slide III ^{3in1}	75	75	100	250
µ-Slide VI $^{0.4}$	75	75	100	250
µ-Slide VI $^{0.1}$	300	300	400	1000
µ-Slide VI flat	75	75	100	250
µ-Slide y-shaped	75	75	100	250
µ-Slide V	75	75	100	250
µ-Slide upright $^{0.7}$	60	60	80	200
µ-Slide Chemotaxis[1)]	45	45	60	150
µ-Slide Chemotaxis[2)]	100	100	133	330
µ-Slide Chemotaxis 3D[1)]	40	40	55	130
µ-Slide Chemotaxis 3D[2)]	70	70	90	230

Open Formats

	Collagen IV	Fibronectin	Poly-L-Lysine	Poly-D-Lysine
µ-Dish $^{35mm, low}$	15	15	20	50
µ-Dish $^{35mm, high 3)}$	15	15	20	50
µ-Dish $^{50mm, low}$	18	18	25	60
µ-Slide 8 well	11	11	15	35
µ-Slide 2x9 well	12	12	17	40
µ-Slide 18 well	12	12	17	40
µ-Slide Angiogenesis	38	38	50	125
µ-Plate 96 well	12	12	15	35
µ-Plate 384 well	24	24	30	70
µ-Chamber 12 well	11	11	15	35
Culture-Insert	18	18	25	60
Culture-Insert StemCell	35	35	47	115

[1)] when coating full chamber
[2)] when coating observation area only
[3)] also valid for glass bottom and ESS versions

The dilutions are calculated using the following coating areas and volumes:

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 V	0.25 per channel	0.5 per channel	30.0 per channel
µ-Slide upright $^{0.7}$	4.35	10.31	250.0
µ-Slide Chemotaxis[1)]	0.96 per chamber	2.40 per chamber	80.0 per chamber
µ-Slide Chemotaxis[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 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-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
Culture-Insert StemCell	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 and ESS version

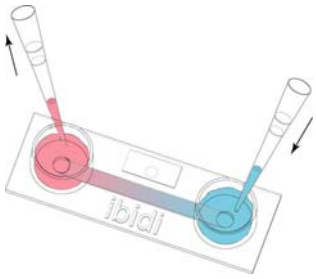
Keep in mind that channel slides are coated on all walls inside the channel. Open formats are coated not only on the growth area but also on the side walls. The coating areas are valid for the exact coating volumes in the table only.

3. Fill the channel or the wells with the coating solution.

Quick dispensing helps to fill the channel slides easier. Work under sterile conditions. Consider that incomplete filling leads to reduced cell growth. Due to hydrophilicity, the ibiTreat surface gets wetted much better than the hydrophobic, uncoated surface. The very small channels are filled more easily by using a small volume syringe with a male Luer tip.

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 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.



7. **Wells or channels are ready to use. Optionally, let dry at room temperature. Attention, some coating proteins 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.