

## **Coating procedures for ibidi $\mu$ -Slides and $\mu$ -Dishes**

For optimized cell adhesion there are different treatments and coatings for the  $\mu$ -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 the ibiTreat surface, uncoated slides have a hydrophobic surface, which must be coated with adhesion factors for the adhesion of most cells.

### **1. Recommended $\mu$ -Slide 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)

Please note that there is no ibiTreat version of  $\mu$ -Slide V.

If you want to do a different coating by yourself, we recommend trying both surfaces, ibiTreat and uncoated.

### **2. Prepare the coating solution**

All coating solutions are calculated for a certain amount of protein per area ( $\mu\text{g}/\text{cm}^2$ ) recommended by the manufacturer's reference.

For Collagen IV: (1.5  $\mu\text{g}/\text{cm}^2$ )

Dilute the Collagen IV (e.g. Becton-Dickinson, mouse tumor, No. 356233) to the desired concentration using in 0.05 M HCl.

For Fibronectin: (1.5  $\mu\text{g}/\text{cm}^2$ )

Dilute the Fibronectin (e.g. Becton-Dickinson, human plasma, 354008) to the desired concentration using PBS (pH 7.2) without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ .

For Poly-L-Lysine: (2  $\mu\text{g}/\text{cm}^2$ )

Dilute the PLL (e.g. 0.01% solution, 100  $\mu\text{g}/\text{ml}$ , Sigma-Aldrich, P4832) to the desired concentration using ultra pure water.

For Poly-D-Lysine: (5  $\mu\text{g}/\text{cm}^2$ )

Dilute the PDL (e.g. Becton-Dickinson, No. 35 4210) to the desired concentration using ultra pure water.

Use the following protein concentrations [ $\mu\text{g/ml}$ ]:

	Collagen IV	Fibronectin	Poly-L-Lysine	Poly-D-Lysine
$\mu$ -Slide I:	75	75	100	250
$\mu$ -Slide VI:	75	75	100	250
$\mu$ -Slide VI flat:	75	75	100	250
$\mu$ -Slide y-shaped:	75	75	100	250
$\mu$ -Dish <sup>35mm, low</sup> :	15	15	20	50
$\mu$ -Dish <sup>35mm, high</sup> :	15	15	20	50
$\mu$ -Dish <sup>50mm, low</sup> :	18	18	25	55
$\mu$ -Slide 8 well:	11	11	15	35
$\mu$ -Slide 2x9 well:	12	12	17	40
$\mu$ -Slide 18 well:	12	12	17	40
$\mu$ -Plate 96 well:	12	12	15	35
$\mu$ -Slide V:	75	75	100	250
$\mu$ -Slide Chemotaxis:	45	45	60	150
$\mu$ -Slide I <sup>0.2</sup> Luer:	150	150	200	500
$\mu$ -Slide I <sup>0.4</sup> Luer:	75	75	100	250
$\mu$ -Slide I <sup>0.6</sup> Luer:	60	60	80	200
$\mu$ -Slide I <sup>0.8</sup> Luer:	38	38	50	125
$\mu$ -Slide Angiogenesis:	38	38	50	125

The dilutions are calculated using the following coating areas:

	Growth area [ $\text{cm}^2$ ]	Approx. coating area [ $\text{cm}^2$ ]
$\mu$ -Slide I:	2.5	5.4
$\mu$ -Slide VI:	0.6 per channel	1.2 per channel
$\mu$ -Slide VI flat:	0.6 per channel	1.2 per channel
$\mu$ -Slide y-shaped:	2.8	5.6
$\mu$ -Dish <sup>35mm, low</sup> :	3.5	4.1
$\mu$ -Dish <sup>35mm, high</sup> :	3.5	4.1
$\mu$ -Dish <sup>50mm, low</sup> :	7.0	7.9
$\mu$ -Slide 8 well:	1.1 per well	2.2 per well
$\mu$ -Slide 2x9 well:	0.4 per minor well	0.55 per minor well
$\mu$ -Slide 18 well:	0.2 per well	0.25 per well
$\mu$ -Plate 96 well:	0.55 per well	2.35 per well
$\mu$ -Slide V:	0.25	0.5
$\mu$ -Slide Chemotaxis:	-	2.4 per chamber
$\mu$ -Slide I <sup>0.2</sup> Luer:	2.5	5.2
$\mu$ -Slide I <sup>0.4</sup> Luer:	2.5	5.4
$\mu$ -Slide I <sup>0.6</sup> Luer:	2.5	5.6
$\mu$ -Slide I <sup>0.8</sup> Luer:	2.5	5.8
$\mu$ -Slide Angiogenesis:	0.12 per well	0.23 per well

Keep in mind that the channel slides are coated on all walls inside the channel. Dishes are also coated not only on the growth area.

## Fill the channel or the wells with the coating solution

Use the following volumes:

μ-Slide I:	100 μl
μ-Slide VI:	30 μl per channel
μ-Slide VI flat:	30 μl per channel
μ-Slide y-shaped:	110 μl
μ-Dish <sup>35mm, low</sup> :	400 μl
μ-Dish <sup>35mm, high</sup> :	400 μl
μ-Dish <sup>50mm, low</sup> :	700 μl
μ-Slide 8 well:	300 μl per well
μ-Slide 2x9 well:	70 μl per minor well
μ-Slide 18 well:	30 μl per well
μ-Plate 96 well:	300 μl per well
μ-Slide V:	10 μl per channel
μ-Slide Chemotaxis:	80 μl per chamber
μ-Slide I <sup>0.2</sup> Luer:	50 μl
μ-Slide I <sup>0.4</sup> Luer:	100 μl
μ-Slide I <sup>0.6</sup> Luer:	150 μl
μ-Slide I <sup>0.8</sup> Luer:	200 μl
μ-Slide Angiogenesis:	10 μl per inner well

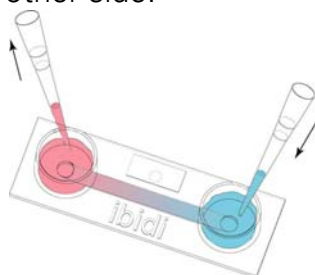
Quick dispensing helps to fill the channel slides easier. Work under sterile conditions. Consider that incomplete filling leads to reduced cell growth. Due to hydrophilicity, the ibiTreat surface gets wetted much better than the hydrophobic, uncoated surface.

### 3. Incubate at room temperature for 60 minutes

### 4. Aspirate the channel or the well volume completely

### 5. Rinse carefully with ultra pure water

For rinsing we recommend to use 10 times the volume of the channel or well. When rinsing a channel slide you can easily add the water into one channel end and simultaneously aspirate it on the other side.



### 6. Wells or channels are ready to use. Optionally, let dry at room temperature.

### 7. 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 perform tests with every lot number. Prepare and use other coating substrates according to the manufacturer's specifications or reference.