## Additional Material Required

*These product references are given for information purposes only. Please contact us if one of these references has been discontinued.*

| Reagents | Supplier and Reference |
|---|---|
| Phosphate-Buffered Saline (PBS) without $Ca^{2+}$ and $Mg^{2+}$<br>Hepes buffer solution 1 M | Invitrogen/Gibco, 14190-094<br>Invitrogen/Gibco, 15630056 |

| Proteins | Supplier and Reference | 2x Protein Concentration and recommended buffer |
|---|---|---|
| Fibronectin from bovine plasma<br>bovin plasma Fibronectin | Sigma F4759-1MG<br>Invitrogen 33010018 | 40µg/mL in PBS |
| Collagen Type I bovine | Sigma, C8919 | |
| Collagen Type I rat tail | BD, 354236 - 100mg | |
| Laminin | Sigma, L2020 – 1mL | |
| Poly-D-Lysine Hydrobromide 70-150KDa | Sigma, P6407 | 80µg/mL in Hepes 10mM |
| Poly-L-Lysine Hydrobromide 15-30KDa | Sigma, P7890 | |
| Fibrinogen Alexa Fluor546 | Invitrogen F-13192 | 2 µg/mL in PBS |

## Recommended Coating Protocol on Activated CYTOOchips™

1. Prepare a solution at the recommended 2x concentration of protein in the corresponding buffer.[1]
2. Transfer a CYTOOchip to a 35mm petri dish (or a 6well plate) with patterned face up.
3. In order to obtain a homogeneous protein coating of the activated micropatterns we recommend that you cover the chip in sufficient buffer to completely wet the surface and then add an equivalent amount of 2x protein solution to the well. (For example, in a 6well dish or 35mm dish, put 2ml of buffer on the chip, check that the chip is completely covered in liquid, then add 2ml of a 2x protein solution in the same buffer).
4. Incubate at room temperature for **two hours**.

[1] for fluorescent micropatterns use a mixture of the adhesion protein and Fibrinogen Alexa Fluor (see table for recommended concentrations)

5. Wash 3 times using PBS. In order to prevent dewetting of the CYTOOchip during the washing step, we recommend to progressively replace the protein solution by successive dilution steps (by first adding fresh buffer and then removing the same volume). **Take care not to dry the CYTOOchip surface during all washing steps.** If the chip is dried during this washing step, protein in solution will adhere on the cytophobic region. See Fig2 for schematic representation of the rinse procedure.

6. The chip coated with laminin should be used immediately
   The chip coated with other protein can be used immediately or air dried and stored at 4°C for several days.

## Troubleshooting

| Problems | Proposed Solution |
|---|---|
| After coating protein on activated CYTOOchips,<br>- cells adhere everywhere, including on the supposedly cytophobic areas<br>Or<br>- cells adhere to micropatterns in only some areas of the chip (not at all in other areas, or everywhere in other areas) | Insufficient liquid in the well can lead to dewetting, and this phenomenon can leave a film of protein all over the chip surface, including the cytophobic area which leads to cells adhering all over the chip.<br>Dewetting can also lead to inefficient micropattern coating |

**Figure 1**: Protein Incubation Step

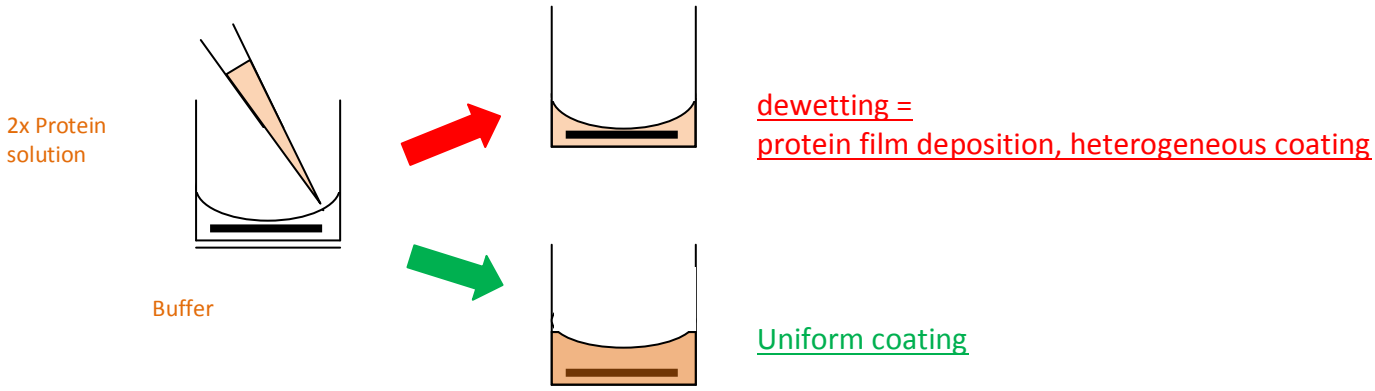


**Figure 2**: Protein washing Step