

	Procedure for Coating CYTOOchips™ Activated with BD Matrigel™ Basement Membrane Matrix	<u>Created :</u> 30/06/2011 MO-Ext-10
---	---	---

I-Goal

BD Matrigel Basement Membrane Matrix is a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in extracellular matrix proteins. Its composition is thus undefined, with a variable total protein concentration of around 10 mg/mL. The major protein component is laminin, followed by collagen IV, heparan sulfate proteoglycans, entactin/nidogen. Here, we propose a protocol to coat CYTOOchips™ with this basement membrane preparation and thus provide adhesive Matrigel™ micropatterns. We recommend the use of CYTOOchips™ pre-coated with PLL (polylysine) to ensure a stronger attachment of the Matrigel to the micropatterns.

For further information:

http://www.bdbiosciences.com/external_files/dl/doc/manuals/live/web_enabled/356234_Guidelines.pdf

<http://www.cytoo.com>

II-Compounds

Product	Supplier and Reference number
BD Matrigel™ Basement Membrane Matrix, 5 ml vial	BD Biosciences 356234
Poly-L-Lysine Hydrobromide 15-30KDa	Sigma, P7890
CYTOOchips™ 20x20 Starter's Activated	CYTOO, 10-900-00-06
DPBS 1X	Invitrogen 14190-094

The day before, prefill sterile eppendorf tubes, tips and pipettes in the refrigerator/cold room. Coat a pre-activated cytochip with PLL (see procedure for coating activated CYTOOchips with PLL).

III- Thaw, aliquot and storage of Matrigel

Thaw the vial overnight at 4°C in the refrigerator or cold room on ice.

Keeping the product on ice, place thawed vial of Matrigel Basement Membrane Matrix in a sterile area ready for aliquoting. Using pre-cooled pipettes, tips, and tubes, pipette the Matrigel, to ensure homogeneity and dispense 400-500 µL per microtube. Store the aliquots at -80°C.

	Procedure for Coating CYTOOchips™ Activated with BD Matrigel™ Basement Membrane Matrix	<u>Created :</u> 30/06/2011 MO-Ext-10
---	---	---

IV- Recommended Coating Protocol

1. Thaw the required number of Matrigel aliquots overnight at 4°C on ice. (Once an aliquot is opened, it can be stored for 3 to 4 days at 4°C). You will need 25µl of Matrigel for each CYTOOchip.
2. Prepare a solution of 1% Matrigel in pre-cooled PBS (using pre-cooled pipettes, tips, and tubes).
E.g. dilute 25µL of Matrigel™ (stock solution) in 2.5mL of pre-cooled PBS. Pipette up and down to homogenize.
3. Transfer a PLL coated CYTOOchip to a petri dish (35 mm) with patterned face up (see procedure for coating activated CYTOOchips with PLL).
4. Add sufficient diluted Matrigel to cover the CYTOOchip (2.5mL is sufficient to cover the chip in a 35mm petri dish or in a 6 well plate).
5. Incubate at 4°C for 2 hours at least (this incubation can also be done overnight).
6. Wash the CYTOOchip without drying it: carefully aspirate the Matrigel solution while adding new cold PBS at the same time. Take care to keep the chip wet at all times. If the chip is dried before diluting extensively the Matrigel solution, Matrigel may adhere on the cytophobic region.
7. Wash with at least 25ml of cold PBS and use the chip immediately.

Suggestion:

Since Matrigel is principally composed of laminin, adhesive Matrigel micropatterns can be immunofluorescently labeled using an anti-laminin antibody (Sigma, #L9393, 1:1000 dilution, incubation 1h).