



Important points for using Matrigel™ overlay on cells

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At CYTOO, we use Matrigel™ for *in-vitro* 3D cell culture of epithelial cells in order to form acini on micropatterns. Here are some important points and recommendations concerning Matrigel that we have learnt the hard way and which are good to know beforehand.

Caring for cells growing in Matrigel :

- 1) Most experiments require that the medium containing Matrigel on cells should be renewed every 4 days. In order not to disturb the cells during the medium change, take off 50% of the volume and add in 50% volume of a 1x concentrated Matrigel (not 2x Matrigel because the previous Matrigel remains).
- 2) During medium changes, It is important that the final volume of culture medium containing cells is not actively mixed or shaken in order to allow Matrigel components to deposit slowly and evenly at the bottom of the well and on the cells.

Manipulation of Matrigel :

- 1) The Matrigel from the stock bottle should be aliquoted according to manufacturer's instructions in to prechilled 1 ml or 0.5 ml eppendorf tubes. Make single use aliquots of 50, 100, 200, and 400 µls into eppendorfs that are sitting on dry ice or a container that has been precooled at -20°C and is sitting on ice.
- 2) Always thaw Matrigel aliquots on ice in a fridge, not just in the fridge because the fridge is at 4°C and this is not cold enough for the Matrigel to remain liquid and instead it will become solid. Ice is at 0°C.
- 3) Always use tips that are precooled for handing Matrigel. We keep a batch of tips in the -20°C.
- 4) Pipette the Matrigel into medium that is from the fridge, not room temperature medium or 37°C heated medium. This ensures that the Matrigel deposits homogenously over the cells in culture.
- 5) It is always preferable to make small enough aliquots such that you use freshly thawed Matrigel for your experiments and for each change of Matrigel containing medium on your cells. However, if you are sure that your particular experiment will not be affected (and this should be tested beforehand) you can avoid throwing away unused medium containing Matrigel by storing the mix in the fridge (not on ice here). In our hands, we can keep medium containing low amounts of Matrigel for up to 7 days. However, past this point the proteins in the Matrigel precipitate out.
- 6) As well as extracellular matrix components, Matrigel also contains several growth factors including TGF-β, EGF, IGF-1, FGF and PDGF which can influence cellular behaviour. It may be important for the interpretation of your experiments to avoid these factors. If so, use Matrigel that is Growth Factor Reduced (GFR). Be aware though that although the level of growth factor is reduced, it is not eliminated completely and especially TGF-β remains, since this is bound to collagen IV and or sequestered in a latent form that partitions with the major components of Matrigel during the purification procedure.



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7) For assays requiring fluorescence detection use phenol red free Matrigel.

8) Matrigel is variable from batch to batch leading to phenotypic variation and proliferation response. Once you have a bottle that works, try to reserve the batch from the supplier. It is worth pretesting and validating each new batch before starting important experiments.