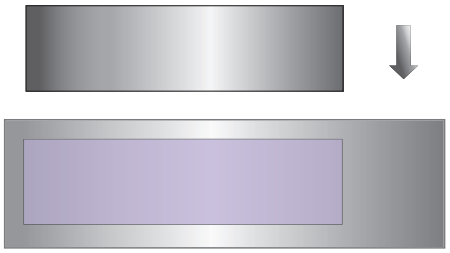
Protocol ♦ Smears & Cytospins

Single cells, such as a typical plasmoblast from a blood smear or other relevant cytological cells, can be identified, cut and isolated with the MMI CellCut Laser Microdissection System. This protocol shows how adherent and cells in suspension are prepared for laser microdissection.

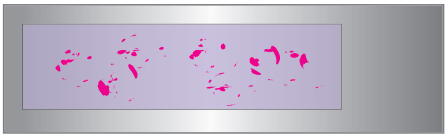
Materials:

- Cell suspension
- PBS-buffer, pH 7.4
- 2 % Agarose
- Centrifuge
- MMI Membrane Slides (PN: 50102, 50103)
- Parafilm
- Pipettes
- Cytocentrifuge
- MMI Support Slide (PN: 50105)
- Cotton swabs
- Poly-L-Lysine solution
- 70 % Ethanol



Prior to centrifuging, place the MMI Support Slide into the frame of the MMI Membrane Slide

Ready for centrifugation



Method:

Cell Suspensions:

1. Trypsinize and/or spin down cells in a centrifuge tube - spin for 10 minutes according to manufacturers recommendation
2. Remove supernatant
3. Wash 2x with PBS pH 7.4 (use RNase-free buffer)
4. Remove supernatant, add 1 ml of heated 2 % agarose (add a drop of Eosin to aid visualization of pellet)
5. Pipette mix the solution using a sterile pipette
6. Using a pipette, transfer the entire sample as one large drop on top of a sheet of Parafilm (RNase-free if required)
7. After the drop has solidified, it can now be treated the same as a biopsy - follow frozen or paraffin embedding protocols and later section into slides

Cell Smears:

1. To enhance adherence, coat MMI Membrane Slides with Poly-L-Lysine
2. Incubate in 0.1 % Poly-L-Lysine solution at 37 °C for at least 1 h (alternatively, gelatine or agarose can be used)
3. Use a cover glass to gently lay your smear on the MMI Membrane Slide
4. Allow to air dry or fix with 70 % Ethanol

Cytospins:

1. Follow general cytospin protocols
2. Use MMI Support Slide (PN: 50105) for centrifugation

Note: Additional coatings (Poly-L-Lysine, Agarose, or Gelatine) are recommended for tissues that are fatty, hard, fibrous, or contain carilage/bone