PROTOCOL
ISOLATION OF LYMPHOCYTES FROM WHOLE BLOOD
For further questions contact: monika.hegi@chuv.ch

This protocol describes isolation of live peripheral blood lymphocytes (PBL) for subsequent use in translational research. The procedure is compatible with modern molecular techniques (e.g. RNA & DNA, and protein isolation for genomics & proteomics) and use of live cells for subsequent in vitro experiments including immortalization by EBV.

- Always wear gloves and glasses for protection.
- Keep area separated from amplified DNA.

METHOD: Isolation of Live Lymphocytes

Preparation of blood with EDTA is best to isolate DNA and RNA (heparin makes complexes with DNA).

Preparation with heparin allows immortalization of lymphocytes with EBV (for unlimited supply of DNA and RNA).

- **ACCUSPIN™ System-HISTOPAQUE®-1077**, Sigma A7054, with respective directions or use the following procedure:

MATERIAL

<table>
<thead>
<tr>
<th>Item</th>
<th>Manufacturer</th>
<th>Catalog number</th>
</tr>
</thead>
<tbody>
<tr>
<td>cryo-tubes (screw top) 2 ml</td>
<td>Nalgene, Cryogenic Vials</td>
<td>5000-0020</td>
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<tr>
<td>cryo-pen (permanent, frost &amp; solvent resistant)</td>
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<tr>
<td>Ficoll separating solution density 1.077</td>
<td>Amersham, Ficoll-Paque</td>
<td>17-1440-02</td>
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<tr>
<td>PBS (phosphate buffered saline)</td>
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<tr>
<td>*FCS (fetal calf serum, or neonatal calf serum)</td>
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<tr>
<td>DMSO (dimethyl sulfoxide)</td>
<td>Fluka</td>
<td>41640</td>
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<tr>
<td>Centrifuge tubes</td>
<td>Eg. Accuspin Tubes (sterile)</td>
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<tr>
<td>-80°C Freezer</td>
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*Optional, for freezing viable lymphocytes
Method:
- 10 ml blood with EDTA as anticoagulant and 10 ml blood with heparin
- Separate lymphocytes and monocytes from other blood components using the Ficoll method:
  - Dilute the blood with sterile PBS 1:1.
  - Add 10 ml of Ficoll in a centrifuge tube, (the proportion between Ficoll and blood should be 1/3 and 2/3, respectively)
  - Carefully pour the diluted blood onto the ficoll solution (the blood must remain on top, do not mix).
  - Centrifuge the tubes 20 min at 1600rpm (350g).
  - Harvest the ring with white blood cells without touching the Ficoll using a sterile pipette tips.
  - Dilute the white blood cells with PBS, then wash them twice in PBS.
  - Resuspend the pellet in 1.5 ml FCS containing 10% DMSO. Put in 2 cryotubes. (For purposes not requiring live lymphocytes (e.g. DNA & RNA isolation) the pellet can be frozen directly)
  - Mark Heparin or EDTA pretreatment on the label.
  - Store PBL at -80°C.

For DNA or RNA isolation (EDTA-prep), thaw tube, pellet lymphocytes, resuspend in appropriate buffer.