



## **Preparation of human platelet-free plasma (PFP)**

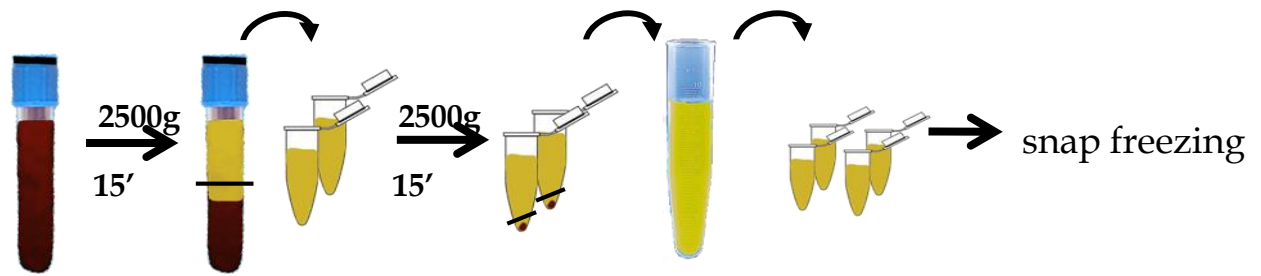
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### Pre-analytical considerations

- Suggested use of ACD-A anticoagulant plastic tubes for blood sampling
- Do not use a needle <21Gauge
- Please apply gentle compression of blood vessels during blood collection
- Please discard the first 2-3mL of blood
- Turn the tube with blood in it 3-4 times upside down gently to mix it with the anticoagulant
- Store the collected blood on room temperature (RT), it must not be put into the fridge!!!
- If the blood collection takes place outside the lab, please pay special attention to prevent shaking during transportation. Place the tubes into stable racks in which they can stand vertically.
- Start sample processing within 1 hour after blood collections (within 2 hours at maximum).

### PFP preparation

1. The first centrifugation should be carried out in Hermle Z206A (Hermle Labortechnik GmbH, Wehingen, Germany) at 2500 g for 15 minutes at RT (acceleration/break: medium).
2. The obtained platelet poor plasma (PPP) should not be removed completely from the pellet, leave ~ 300-500µl on top of it. The removed PPP is pipetted into Eppendorf tubes (1-4 tubes depending on the volume of the PPP plasma),
3. Spin down the Eppendorf tubes again at 2500 g for 15 minutes (Eppendorf 5417R, Eppendorf AG, Hamburg, Németország).
4. Transfer the supernatant (PFP) into a 6 mL polypropilene (PP) tube leaving ~ 50-75µl plasma on top of the pellet (to ensure that the pellet is not stirred up).
5. Pipet the PFP within the tube 3x to ensure that it is mixed properly (to ensure homogeneity of the PFP)
6. Distribute it into 250-500 µl aliquots (n=6-8).

### **Storage of PFP samples**

Snap freeze the samples in liquid nitrogen and store that at -80°C until use.

### **References:**

Based on the standardizing protocol of the International Society on Thrombosis and Hemostasis (ISTH) [Standardization of pre-analytical variables in plasma microparticle determination: results of the International Society on Thrombosis and Haemostasis SSC Collaborative workshop.

Lacroix R, Judicone C, Mooberry M, Boucekine M, Key NS, Dignat-George F; The ISTH SSC Workshop. *J Thromb Haemost.* 2013 Apr 2. doi: 10.1111/jth.12207.]

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