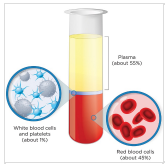

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Plasma Preparation

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

Protocol for preparing plasma for metabolomics studies / GWAS

PROTOCOL CITATION

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MATERIALS

NAME	CATALOG #	VENDOR
10mL syringe	75846-756	VWR international Ltd
General Use and PrecisionGlide Hypodermic Needles, 18 G x 1.5 in. (38mm); Regular bevel; Pink	148265D	Thermo Fisher
Denville Posi-click 1.7ml Eppendorf Tubes	C2172	
Microvette Blood Collection w/inner tube Edta	NC9976871	Fisher Scientific
BD Vacutainer™ Plastic Blood Collection Tubes with K2EDTA: Hemogard™ Closure	BD 367862	Fisher Scientific

BEFORE STARTING

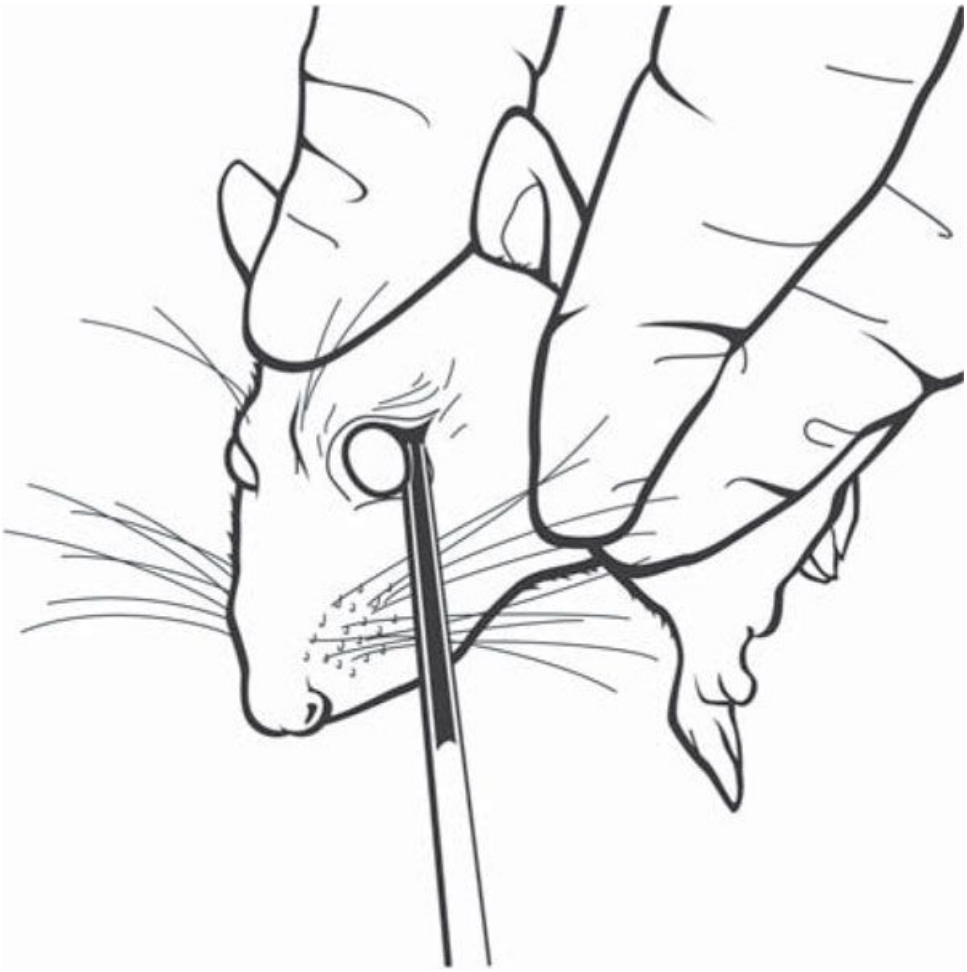
Prepare 2ml eppendorf tubes to spin down whole blood
Ensure that blood is taken using tubes coated with EDTA (heparin is not as good as it causes hemolysis)
Always keep blood at room temperature

1 **Collect blood into the EDTA coated microvette tube**.
Once spun, about half will be plasma, half will be whole blood. **Room temperature**

For **initial harvest** of blood, we use retro-orbital blood sampling. This is done under general anesthesia (isofluorane)

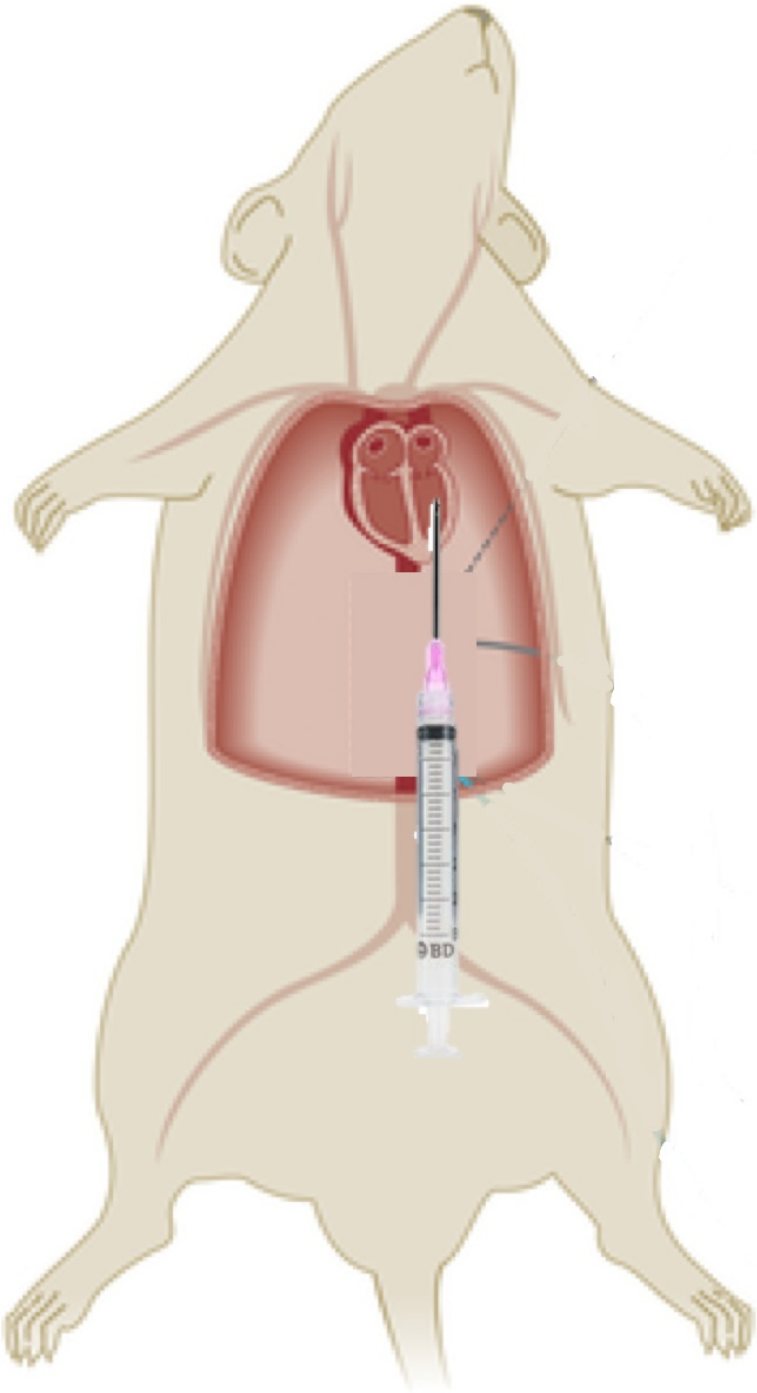
- The tip of the capillary tube is placed at the medial canthus of the eye under the nictitating membrane.
- A short thrust past the eye and the capillary will enter the sinus membrane which will have slight resistance.
- Apply slight pressure to the membrane and the blood will begin to flow into the capillary.

- Collect 200-400ul, then remove the capillary tube from under the eye and apply slight pressure to stop bleeding.
- Invert the tube 2-3 times to ensure the EDTA is evenly mixed in the sample, then move to step 2.



For the **final harvest** of blood, we collect during the dissection of the animal. This allows us to directly collect blood from the circulatory system. The animal is first anesthetized with carbon dioxide, and then the heart is exposed.

- With an 18 gague needle, puncture slightly to the right side (animals left) of the apex of the heart.
- Apply slight backward pressure to the syringe to extract the blood.
- 2-3 mls is sufficient. The blood is transferred from the syringe to an EDTA coated tube.
- Invert the tube 2-3 times to ensure the EDTA is evenly mixed in the sample, then move to step 2.

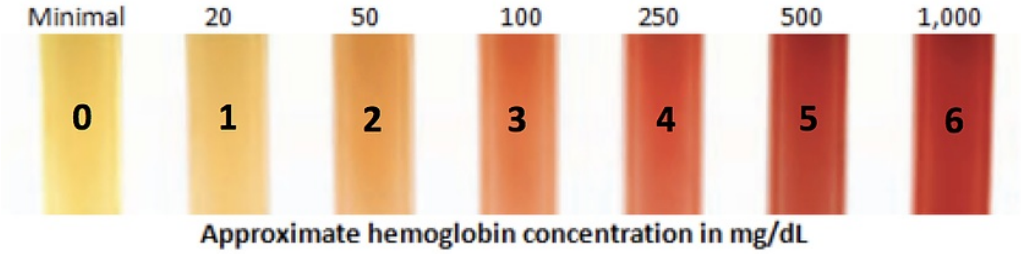


2 Maintain the whole blood sample at room temperature, then aliquot the sample into a single tube or multiple tubes depending on the sample size. Freezing or extended time on ice will result in hemolysis.

3 Spin the whole blood samples for 10 min at 2000 g at RT to pellet the erythrocytes. **Room temperature** 10m

4 Immediately transfer 500 µL aliquots of the plasma in to fresh 2 mL labeled eppendorf tubes using a pipette with appropriate filter tip. Care should also be taken to avoid taking any red blood cells over into the plasma. It is best to err on collecting less than contaminating with red blood cells.

5 Visualize the quality of the plasma on the hemolysis scale below and mark the color of the plasma according to the scale 0-6 on the GWAS Blood collection sheet. It is optimal that the score is less than "1", take extra notes if there is excessive hemolysis, or clotting in the sample.



Hemolysis Scale

6 Special care should be made to ensure that the identification on each vial is legible. Make sure that the pen you use is permanent ink and will not wash off upon thawing.

Make sure the tube is labeled with an "A" for BSL, a "B" for final blood

7 Samples should be stored at -80 °C until analysis and all transportation should be carried out under dry ice.