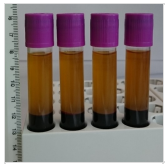

Version 9

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plasma preparation exRNAQC V.9

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1 Works for me dx.doi.org/10.17504/protocols.io.t3reqm6

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ABSTRACT

This protocol describes how to prepare different plasma fractions from venous blood draw (10 ml) according to the extracellular RNA quality control study.

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KEYWORDS

plasma, platelet-rich plasma, PRP, platelet-poor plasma, PPP, platelet-free plasma, PFP, platelets

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GUIDELINES

- In the Center for Medical Genetics Gent (CMGG), plasma preparation is performed in the blood lab. Guidelines on how to handle blood samples and how to work in this lab are described in H5.3-OP2-B1.
- Take pictures of tubes (optional) on a white background, upright position.

Materials

1
- Liquid nitrogen
- Pipettes and filter tips
- Centrifuge with swinging bucket rotor (centrifugation speed up to 2500 g (rcf)) and soft brake function, and buckets for blood collection tubes and conical 15 ml tubes
- Mini centrifuge (for centrifugation of 1.5 ml tubes)
- Disposable lab coat
- Nitrile powder-free gloves
- VERSI-DRY Lab Table Soakers (Nalgene, cat. no. 62080-00)
- Safe-Lock cup DNA LoBind 1.5 ml PCR clean (Eppendorf, cat.no. 022431021)
- Conical 15 ml polypropylene tube (Cellstar, cat.no. 188271)
- Racks for different types of tubes

Centrifugation step 1: 20 min at 400 g (rcf) - preparation of PRP

2
- Invert tubes 5 times before centrifugation.
- Spin tubes for 20 min at 400 g (rcf) (without brake), at room temperature. Note time point of centrifugation start.
- Pipette platelet-rich plasma (PRP) carefully into a new collection tube, leave ± 0.5 cm above the buffy coat (do not disturb the buffy coat).
- Invert the PRP tube before aliquoting. Aliquot the PRP into LoBind tubes, snap freeze in liquid nitrogen and store at -80 °C (note time point of snap freeze), and/or continue to prepare platelet-poor plasma (PPP) and/or platelet pellet. If this is the end point of your plasma preparation, measure hemolysis (see protocols.io 'Hemolysis measurement of plasma/serum samples with Nanodrop ND1000'). If you are not sure if this is the end fraction that will be used in your final experiment, also measure hemolysis.

Centrifugation step 2: 10 min at 800 g (rcf) - preparation of PPP and platelet pellet

3 **Preparation of platelet pellet**
- Pipette 250 µl PRP into a LoBind tube.
- Spin the PRP for 10 min at 800 g (rcf) (without brake) to obtain a platelet pellet, at room temperatue.
- Carefully remove the plasma and snap freeze the platelet pellet in liquid nitrogen, and store at -80 °C (note time point of snap freeze).

Preparation of PPP
- Spin the remaining PRP for 10 min at 800 g (rcf) (without brake) to obtain platelet-poor plasma (PPP), at room temperature.
- Pipette PPP carefully into a new collection tube, leave ± 0.5 cm above pellet (do not disturb pellet).
- Invert the PPP tube before aliquoting. Aliquot the PPP into LoBind tubes, snap freeze in liquid nitrogen and store at -80 °C (note time point of snap freeze), and/or continue to prepare platelet-free plasma (PFP). If this is the end point of your plasma preparation, measure hemolysis (see protocols.io 'Hemolysis measurement of plasma/serum samples with Nanodrop ND1000'). If you are not sure if this is the end fraction that will be used in your final experiment, also measure hemolysis.

Centrifugation step 3: 15 min at 2500 g (rcf) - preparation of PFP

4
- Spin the PPP for 15 min at 2500 g (rcf) (without brake) to obtain platelet-free plasma (PFP), at room temperature.
- Pipette PFP carefully into a new collection tube, leave ± 0.5 cm above pellet (do not disturb pellet).
- Aliquot the PFP into LoBind tubes, snap freeze in liquid nitrogen and store at -80°C (note time point of snap freeze). If this is the end point of your plasma preparation, measure hemolysis (see protocols.io 'Hemolysis measurement of plasma/serum samples with Nanodrop ND1000'). If you are not sure if this is the end fraction that will be used in your final experiment, also measure hemolysis.

Notes

5 PRP, PPP and PFP contain approximately 125, 10 and less than 1% of platelet concentration in whole blood (~ 300,000 platelets/µl), respectively.

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