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plasma preparation_exRNAQC V.7

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1 Works for me

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ABSTRACT

This protocol describes how to prepare plasma from venous blood draw.

ATTACHMENTS

plasma preparation.pdf

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PROTOCOL CITATION

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plasma

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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 (optional) on a white background, upright position

MATERIALS

NAME	CATALOG #	VENDOR
Liquid Nitrogen		
Pipettes		
Safe-Lock cup DNA LoBind 1,5ml PCR clean	A08970	Eppendorf
Cryotube	10674511	Thermo Fisher Scientific

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NAME	CATALOG #	VENDOR
Centrifuge with swinging bucket rotor (centrifugation speed up to 2500 g (rcf)) and buckets for blood collection tubes and conical 15 ml tubes		
Disposable lab coat		
Nitrile powder-free gloves		
Nalgene® 62080-00 VERSI-DRY® Lab Soaker Bench Protector Mat, White Standard Absorbency	NAL-62080-00	Thermo Fisher Scientific
Filter tips		
Racks for the different types of tubes		
Conical tube, 15 ml	188271	greiner bio-one

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Centrifugation step 1: 20 min at 400 g (rcf)

- 1 Invert tubes 5 times before centrifugation
 - Spin tubes for 20 min at 400 g (rcf) (without brake), at room temperature. Note time point of start of centrifugation
 - 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 cryovials or 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)

Centrifugation step 2: 10 min at 800 g (rcf)

- 9 Spin the 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)
 - Optional: If platelets need to be collected, resuspend pellet in remaining volume above pellet, transfer to 1.5 ml tube, and spin again for 10 min at 800 g (rcf) (without brake), remove volume and snap freeze pellet in 1.5 ml tube. Note time point of snap freeze
 - Invert the PPP tube before aliquoting. Aliquot the PPP into cryovials or 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)

Centrifugation step 3: 15 min at 2500 g (rcf)

- 3 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 cryovials or LoBind tubes, snap freeze in liquid nitrogen and store at -80°C (note time point of snap freeze)