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Dry blot spot cards - Processing plasma EDTA from Whole Blood

 In 1 collection

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details the processing of plasma EDTA from whole blood sample.

Guidelines

The protocol needs prior approval by the users' Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s) as applicable.

Safety warnings

 The protocol needs prior approval by the users' Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s) as applicable.

Before start

The protocol needs prior approval by the users' Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s) as applicable.



Blood Spot samples

4h 30m

- 1 Gently invert the purple cap tube(s) 180° and back 8-10 times immediately after collection. Allow 00:20:00 - 00:30:00 to cool down prior to sample processing. 30m
- 2 Aliquot 5 drops of 75 µL of whole blood (EDTA) onto Whatman 903 filter paper. Set the rest of the whole aside.
- 3 Dry blood spots for a minimum of 04:00:00 at Room temperature . 4h
- 4 Store dried sample cards in sealed plastic bags at -80 °C with a desiccant.

Procedure

20m

- 5 The tube with remaining blood following the dried blood spot should be gently inverted 180° and back 8-10 times.
- 6 Centrifuge the tube at 1200 x g, 4°C, 00:10:00 **NO BRAKE.** 10m
- 7 After centrifugation there should be a clear separation between the red blood cells at the bottom and the plasma at the top.
- 8 Use the pasteur pipette provided or a micropipette to carefully aliquot the EDTA plasma layer into a 15L falcon tube. Approximately 3 mL / 3000 µL of the plasma from the purple cap tube into the falcon tube.

Note

Do not to disturb the red blood cell layer and set the tube aside. If the sediment is disturbed the centrifugation step must be repeated.



9 Place the falcon tube in the centrifugation at  1800 x g, 4°C, 00:10:00 **NO BRAKE.**

10m



10 From the red blood cell layer, use a micropipette to carefully aliquot  500 µL of the red blood cells from the purple cap tube into the 4-5 × 1ml cryovials (1000µl into each tube) labelled as RBC.



11 Retrieve the falcon tube EDTA plasma layer from the centrifuge. Use a micropipette to carefully aliquot  300 µL of the plasma from the falcon tube into the 2-3 × 1ml cryovials ( 1000 µL into each tube) labelled as EDTA-PLA.

Note

Do not to disturb the pellet at the bottom of the tube. If the sediment is disturbed the centrifugation step must be repeated.

12 Place plasma samples in  -80 °C .