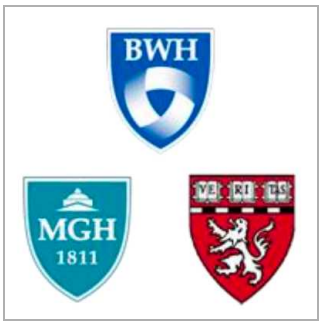

NOV 21, 2022

WORKS FOR ME 1

Whole Blood, Plasma, and Buffy Coat Processing

COMMENTS 0

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.6qpvrdp8pgmk/v1

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Daniel's workspace

Daniel El Kodsi

ABSTRACT

This protocol explains the Standard Operating Protocol for processing whole blood, plasma, and buffy coat.

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PROTOCOL CITATION

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COLLECTIONS

BIOSPECIMENS SOPs

KEYWORDS

whole, blood, plasma, buffy coat, ASAPCRN

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OWNERSHIP HISTORY

Feb 18, 2021	 Liz Brydon	Protocols.io
May 03, 2021	 Yuliya Kuras	
May 05, 2021	 Yuliya Kuras	
Oct 03, 2022	Daniel El Kodsi	

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PARENT PROTOCOLS

Part of collection
BIOSPECIMENS SOPs

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GUIDELINES

FREEZER STORAGE

Freezers are divided into 4 shelves, with 6 racks per shelf, and 24 boxes that can be held in each shelf. In total, 576 boxes, approximately 2,160 sample sets, can be stored in one -80°C freezer. The first three shelves are designated by visit number: Shelves A1-6 (top shelf) house samples from enrollment visits, shelves B1-6 (2nd shelf) house samples from the 1st year follow-up, and shelves C1-6 (3rd shelf) house samples from the 2nd year follow-up. Shelves D1-6 contain packed red blood cell tubes (PRBC), DNA, and RNA, extracted from blood as described in the protocols above. CSF is designated between two freezers in selected racks. Freezer storage and transactions of samples are recorded in the Freezerworks Inventory software.

MATERIALS TEXT

MATERIALS:

1. Low retention 1.5 mL tubes (Fisher Scientific, Cat #02-681-320)
2. Low retention pipette tips

a. 1000 mL low-retention tips (Bio Plastic, Inc., Cat #3606SRS)
b. 200 uL low-retention tips (Molecular BioProducts, Cat #3932-05)
3. Freezerbonz labels (Fischer Scientific, Cat #22500521)

Be sure to use only the low retention tubes and low retention tips for blood processing!
To save time: Print labels, label tubes, and set up subject entry in freezerworks before samples arrive!

SAFETY WARNINGS

Please refer to Safety Data Sheets (SDS) for health and environmental hazards. Gain all required consent and experimental

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approvals before beginning any procedures.

BEFORE STARTING

Be sure to use only the low retention tubes and low retention tips for blood processing!
To save time: Print labels, label tubes, and set up subject entry in freezerworks before samples arrive!

WHOLE BLOOD, PLASMA, AND BUFFY COAT PROCESSING Q/C GOAL

1. Time from blood draw to -80°C freezing of aliquots ≤ 4 hours

Whole Blood, Plasma, and Buffy Coat Processing

1 **For PAXgenes:**

1. Label the collection tubes with the sample ID as appropriate.
2. Place aliquot tubes on dry ice prior to procedure so they are pre-cooled.

2 *Before centrifuging the 10ml BD purple top tube*, pipette 550 µL aliquots of whole blood into two Eppendorf tubes labeled "WB-01 and WB-02."

3 Place the 10 ml BD purple top and the two 6 ml BD purple tops into the blue racks and, using the Eppendorf centrifuge 5810R set to 2000 rcf, 25°C, 00:05:00.

4 **10 ml BD purple top tube.** After centrifugation, pipette 500 µL plasma into Eppendorf tubes labeled "PL-01, PL-02, etc" (8-10 tubes depending on the sample). Avoid introducing bubbles into the tube, as it will make it harder to pipette the plasma. Stop taking plasma when the buffy coat begins to enter the pipette tip. If the amount of plasma in the tip is less than 500 uL, place the remaining amount into the last plasma tube labeled "PL-16." Switch to a 100 uL pipette and remove any excess plasma that is sitting above the buffy coat. Place this excess plasma in the last plasma tube labeled "PL-16."

4.1 With the 100 uL pipette, pipette approximately 200-250 uL of buffy coat into one Eppendorf tube labeled "BC-01." (Try to avoid pipetting up the pellet as much as possible; however, some pellet will be unavoidably pipetted up with the buffy coat.) If more than 250 uL of buffy coat can be extracted, place the remaining volume of buffy coat into the other buffy coat tube labeled "BC-02."

5 **1st 6 ml purple top tube.** After centrifugation, pipette 500 µL plasma into the remaining, empty plasma tubes. Avoid introducing bubbles into the tube, as it will make it harder to pipette the plasma. Stop taking plasma when the buffy coat begins to enter the pipette tip. Switch to a 100 uL pipette and remove any excess plasma that is sitting above the buffy coat. Any excess plasma can be equally distributed amongst the 16 plasma tubes.

5.1 With a 100 uL pipette, pipette approximately 200-250 uL of buffy coat into the tube labeled "BC-02."

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6 **2nd 6 ml purple top tube.** After centrifugation, pipette 500 µL plasma into the remaining empty plasma tubes. Avoid introducing bubbles into the tube, as it will make it harder to pipette the plasma. Stop taking plasma when the buffy coat begins to enter the pipette tip. Switch to a 100 uL pipette and remove any excess plasma that is sitting above the buffy coat. Any excess plasma can be equally distributed amongst the 16 plasma tubes

6.1 With a 100 uL pipette, pipette approximately 200-250 uL of buffy coat into the tube labeled "BC-03."

Whole Blood, Plasma, and Buffy Coat Storage

7 Split each set of tubes into two boxes and label according to their H number. The various aliquots will be placed into each box and scanned as follows:

1. Box 1 (labeled with a * and stays here): Whole Blood 01, Plasma 01-08, and Buffy Coat 01-03
2. Box 2 (goes to backup freezer): Whole Blood 02, and Plasma 09-16
3. PRBC (placed in designated box in -80C)

The number of aliquots per type of aliquot is dependent upon the sample volume. If an uneven number of aliquots per type of aliquot are produced, then more of the aliquots will be placed in Box 1. (ex: 9 plasma aliquots total = 5 plasma in Box 1 and 4 plasma in Box 2).

8 Place aliquots into the -80 °C within 4 hours of the blood draw.

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