

Insert Institution Department (if applicable) Division (if applicable) Biorepository (if applicable)	Standard Operating Procedure Collection of EDTA Plasma and Buffy Coats	
SOP Repository Banking	Blood Processing for Biobank Network Exchange	
Version (insert #)	Effective Date:	Page 1 of 3
Revision:	Revision Date:	Review Period (insert # yrs)
Authored by:	Approved By:	Date:

1.0 Purpose

This document describes the process for the collection of human plasma from EDTA-anticoagulated blood.

2.0 Scope

These guidelines apply to personnel intending to cryopreserve plasma and/or buffy coats.

3.0 Requirements:

3.1. General Requirements

All specimens will be treated as potentially hazardous. Personal protective equipment (lab coats, gloves and eye protection) must be worn at all times when handling specimens. This includes during the removal of the rubber stopper from blood tubes, centrifugation, pipetting, disposal of contaminated tubes, and cleanup of any spills. Tubes, needles and pipets must be properly disposed of in biohazard containers in accordance with institutional requirements.

It is important to take steps to prevent hemolysis in samples. Blood collection with the BD Vacutainer system is recommended. If a needle is used, a 21-gauge needle is recommended.

3.2. Equipment:

3.2.1 Centrifuge with swinging bucket rotor (times and rcf will need to be adjusted if a fixed angle rotor is used)

3.2.2 -80°C Freezer

3.2.3 Biosafety Cabinet

3.2.4 Pipette Aid

3.3. Materials: (see supply list at end for additional details)

3.3.1 1.2ml, self-standing cryovials and caps

3.3.2 15ml and 50ml Sterile, Polypropylene, Conical, Centrifuge Tubes

3.3.3 Sterile pipettes and/or transfer pipets

4.0 Method:

4.1 After collection via venous blood draw, gently mix the blood by inverting the tube 8-10 times. Store vacutainer tubes upright at 4°C or on ice until centrifugation. Blood samples should be centrifuged within four hours of blood collection.

4.2 Centrifuge the blood samples in a swinging bucket rotor for 10-20 minutes at 1300-1750 rcf at room temperature or 4° degrees C. Note: use <1300 rcf if spinning glass tubes in a fixed angle rotor. 15 min at 1300 rcf will work for most applications and tube styles/volumes. Check for specific guidelines from the manufacturer of the collection tubes.

4.3 After centrifugation, plasma layer will be at the top of the tube. Mononuclear cells and platelets will be in a whitish cell layer called the “buffy coat” just under the plasma and above the red blood cells (additional processing of these cell fractions is optional).

4.4 Carefully collect plasma layer with an appropriate transfer pipette without disturbing the buffy coat layer. It may be helpful to transfer the plasma to a 15 or 50 ml conical tube; plasma from multiple tubes of the same subject should be combined before aliquoting. Aliquot 100µl – 500 µl (insert applicable volume) in labeled cryovials. Close caps tightly and place on ice or immediately freeze. This process should be completed within 1 hour of centrifugation.

4.5 If Buffy Coat cells are desired, remove after plasma has been collected. Typically, 0.5-1.0 ml is collected into one storage tube. A small volume of plasma and/or red blood cells may be collected along with the buffy coat, without negatively impacting the buffy coat cells. This buffy coat is suitable for gDNA extraction. If PBMCs are desired, further purification and storage in a suitable freezing medium is necessary. (see separate SOP; the remaining plasma, buffy coat cells and red blood cells will be used).

4.6 Check that all aliquot vial caps are secure and that all vials are labeled. Place aliquots upright in specimen box or rack in -80°C or colder freezer. All specimens should remain at -80°C or colder and will be transported frozen on dry ice only.

Record/Data Points (**Use Barcode if possible to facilitate sample tracking**)

1. Hemolysis or lipemia in the sample
2. Date and time of blood collection
3. Number and volume of aliquots prepared
4. Date and time plasma transferred into -80°C
5. Date and time of shipping (if applicable)
6. Any freeze-thaw that occurs with a sample for any reason
7. Any variations or deviations from the SOP, problems, or issues

Label Cryovials

1. Subject ID
2. Subject initials (if appropriate; may be an identifier)
3. Date of collection

4. Visit date (if applicable; may be an identifier. Visit number may be desired instead.)

Supplies

1. EDTA Blood Collection Tubes (for example, BD Vacutainer catalog # 366450)
2. Centrifuge with swinging bucket rotor (different times and rcf will be needed for fixed angle rotors)
3. 15 ml and/or 50 ml polypropylene conical tubes (for example, Corning 430052, Fisher catalog #05-538-53D)
4. Sterile cryovials with writing surface (for example Simport T311-2 or Fisher #05-669-57)
5. 2ml, 5ml, and 10ml pipettes (for example, Fisher cat #13-678-11C, 13-678-11D, 13-678-11E)
6. Disposable transfer pipettes (for example, Fisher cat #13-711-20)
7. Small ice bucket
8. Biohazard waste container suitable for human blood; sharps container if glass collection tubes are used
9. Appropriately sized racks and freezer storage boxes.
10. Bleach and/or 70% EtOH.
11. Paper towels or wipes
12. Gloves