Plasma Isolation from Lavender-Top (EDTA) Blood Collection Tubes

Plasma is the liquid component of blood that holds blood cells in suspension. Though water is the major constituent, it also contains electrolytes, hormones, and enzymes. Other important constituents in plasma are antibodies, proteins such as albumin, and clotting factors including fibrinogen.

Tubes coated with an anticoagulant (e.g., EDTA, heparin, or sodium citrate) are used for plasma isolation. Immediately after blood collection, the tubes are inverted several times to ensure proper mixing of anticoagulant with whole blood. Since K2-EDTA is best at preserving cellular components (e.g., DNA) and morphology of blood cells at room temperature, it is the anticoagulant of choice for hematological and molecular testing. Plasma containing K2-EDTA may not be used to measure analytes such as potassium or divalent cations such as calcium. Blood plasma obtained from lavender-top tubes is commonly used for the following applications:

- **Antibody testing**
- **Liver panel test (total protein and albumin)**
- **Blood glucose measurement**
- **Polymerase chain reaction (PCR) assay for infectious disease screening**

Here we describe a step-by-step protocol for isolation and collection of plasma from tubes with K2-EDTA anticoagulant.

Reagents and Materials

- **Venous blood collected in Lavender-Top BD Vacutainer™ K2-EDTA tubes**
- **Pipettes and pipette tips**

Equipment

- **Bench-top centrifuge with swing-out rotor and appropriate carriers**
- **Biosafety laminar flow cabinet (Class II) cabinet**

Procedure

1. Measure and record the estimated total blood volume collected. Keep tubes in an upright position at room temperature (18 °C to 25 °C) until centrifugation.
2. Centrifuge the tubes at 800–1,000 x g at room temperature (18 °C to 25 °C) for 10 minutes with the BRAKE OFF to avoid disrupting the density gradient during deceleration.
3. The following layers should be visible after centrifugation (from top to bottom, **Figure1**):
   - **Yellowish plasma layer (supernatant)**
   - Greyish-white buffy coat containing leukocytes
   - Deep red layer of erythrocytes

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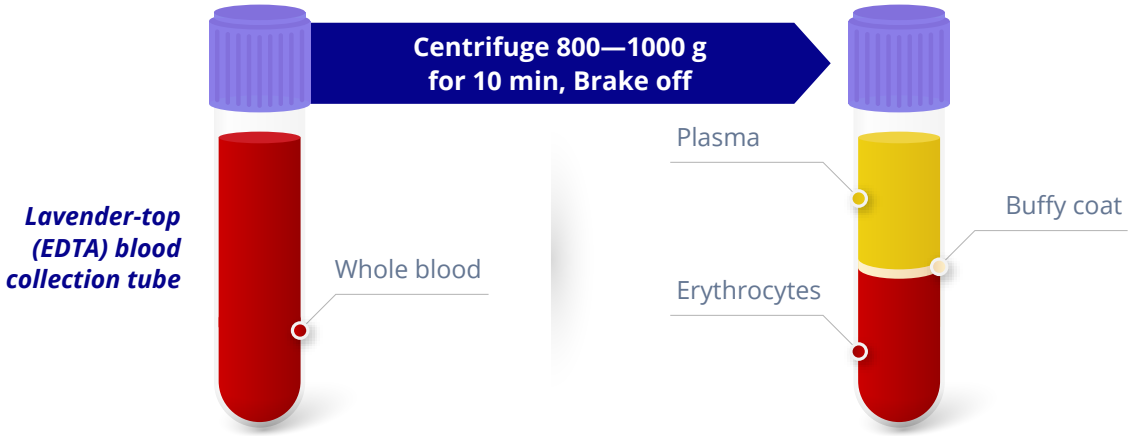

FIGURE 1. **Plasma isolation by density gradient centrifugation**

4. Using a pipette, aliquot 1 mL of plasma into 2 mL cryovials for storage or transportation. Samples should be maintained on ice at 2 °C to 8 °C during handling.

   Note: Do not disturb the buffy coat layer while collecting plasma.

5. Store the cryovials containing plasma at -80 °C.

   Note: Avoid multiple freeze-thaw cycles of plasma aliquots as temperature variations can negatively impact sample quality.

Troubleshooting

Problem	Potential Solutions
Lack of distinct layers after centrifugation	▪ Perform centrifugation with brakes off. Deceleration disrupts the formation of distinct layers. ▪ Weigh tubes to ensure rotors are appropriately balanced. This avoids excessive shaking during centrifugation.
Pink or reddish tinge to plasma	▪ *In vivo* or *in vitro* hemolysis of red blood cells may impart a pink or reddish tinge to plasma. *In vivo* hemolysis may be attributed to diseases such as hemolytic anemia. Further investigation of the patient's clinical history may be warranted. ▪ *In vitro* hemolysis may occur during sample collection, handling, and storage. Ensure samples are maintained at 2 °C to 8 °C during handling and stored at -20 °C or below.