



To measure circulating Transforming Growth Factor-beta 1 (TGF- β 1) accurately, preparing platelet-poor plasma (PPP) using a double-centrifugation method is crucial. The technique minimizes platelet contamination, which is essential because platelets are the body's main carrier of TGF- β 1, storing 40 to 100 times more than other cells.

A single centrifugation spin leaves residual platelets that can release their stored TGF- β 1, artificially inflating measurements and leading to inaccurate results. Therefore, the double-spin procedure provides a more reliable sample.

Platelet-poor plasma (PPP) double-centrifugation protocol

This protocol is based on standard procedures used for coagulation and growth factor analysis.

Materials needed:

Collection tubes: Sodium citrate (light blue top) tubes are the standard anticoagulant for plasma separation. The blood-to-anticoagulant ratio must be 9:1. Ensure tubes are filled completely.

Plastic pipettes: Use only plastic pipettes to transfer the plasma. Glass can activate platelets.

Transfer tubes: Plastic aliquot tubes for the separated plasma.

Centrifuge: A refrigerated, calibrated centrifuge is recommended.

Freezer: A non-self-defrosting freezer at -20°C or -80°C for storage.

Step 1: First spin (separating plasma from cells)

Collect blood into sodium citrate tubes, ensuring the proper fill volume.

Within one hour of collection, centrifuge the balanced tubes.

Settings: Spin at 1500 g for 15 minutes. Alternatively, 3000 RPM for 10 minutes is also used depending on the specific centrifuge. A refrigerated centrifuge is preferred to prevent platelet activation.

After the spin, the tube will show three layers: red blood cells at the bottom, a thin "buffy coat" of white blood cells and platelets in the middle, and plasma at the top.

Step 2: Transfer and second spin (removing remaining platelets)

4. Using a plastic pipette, carefully transfer the top three quarters of the plasma into a new, labeled plastic aliquot tube.



* Crucial: Do not disturb the buffy coat or the cellular layer. Leave approximately 0.5 mL of plasma behind to avoid aspiration of any cells.

5. Re-balance the tubes and centrifuge the transferred plasma a second time, using the same settings as the first spin.

* Settings: 1500 g for 15 minutes.

Step 3: Harvest and storage

6. Following the second spin, use a new plastic pipette to carefully transfer the top 80–90% of the newly spun plasma into a final, labeled plastic aliquot tube.

* Crucial: Again, leave the very bottom of the plasma behind, as any remaining platelets will form a small button at the bottom of the tube.

7. Check the final PPP for any visible hemolysis or clots before proceeding.

8. Immediately freeze the aliquoted PPP at -20°C or, for long-term storage, at -80°C.

Scientific rationale for the double spin

The double spin protocol is standard practice for preparing PPP for coagulation studies, and the principles are equally applicable for sensitive growth factor analysis like TGF- β 1.

Platelet activation: Studies have shown a strong correlation between platelet concentration and TGF- β 1 levels, as well as a significant increase in TGF- β 1 in platelet-rich plasma (PRP) compared to plasma. Inadequate centrifugation leaves behind platelets that can release their stored TGF- β 1, leading to falsely high measurements.

Standardization: Using a standardized double-centrifugation protocol ensures consistency in sample preparation, leading to more reliable and reproducible results for TGF- β 1 analysis.

For extremely low levels of TGF- β 1, some researchers have explored adding prostaglandin E1 (PGE1) during collection to inhibit platelet activation, although this is not a standard practice for routine PPP preparation.