

CAREprp Instructions:

1. Draw 2ml of ACD-A or Sodium Citrate anticoagulant into the included 30ml syringe/18G blunt fill needle. Use the included butterfly to gently draw 23ml of blood into the syringe (for a total volume of 25ml). The tip at the end of the butterfly tube pulls off so that it'll connect to the syringe/Luer Lock. Connect the butterfly to the syringe.
2. Ensure that the bottom chamber is open to the 15mm mark. Adjust by gently rotating the bottom collar clockwise until the top of the internal piston is at the 15ml mark.
3. Gently close the top collar (by turning clockwise) without overtightening.
4. Open the outer port cap. Gently transfer 23ml of blood + 2ml of anticoagulant by attaching the syringe/Luer Lock (no needle) onto the outer port. Reseal the outer port by closing its cap.
5. Open the top chamber by turning the top collar exactly $1 \frac{1}{3}$ turns counterclockwise. $1 \frac{1}{2}$ turns is too much. It might be easier to visualize $1 \frac{1}{4}$ plus a little.
6. Place the CAREprp tube and the counterweight tube (with 25ml of water) into the centrifuge and spin for 3 minutes at 3200 rpms - centrifuge settings are automatic in the PRP mode. Note: As above - open the top chamber of the counterweight tube $1 \frac{1}{3}$ turns and raise the piston in its bottom chamber to 15ml. Also, close the top collar of the counterweight tube after the first spin to lesson evaporation (for next spins).
7. Carefully remove the tube. The red blood cells will be in the bottom chamber. The buffy coat sits immediately on top of the red blood cells. Slowly raise the red blood cells and buffy coat with a firm grip on the tube by **slowly** turning the bottom collar clockwise to the desired position. Your options are:
 - **Leukocyte-Rich PRP** - position buffy coat into the top chamber
 - **Monocyte-Rich PRP** - position half of the buffy coat in the neck; retrieving monocytes and lymphocytes with minimal neutrophils
 - **Leukocyte-Poor PRP** - trap entire buffy coat in the neckLetting the tube sit for a moment will allow any undesired red blood cells that may have entered into the top chamber to settle down into the red blood cell layer.
8. Once the buffy coat has been positioned, turn the top collar clockwise to seal off the connection between the top and bottom chambers - the separator contained within the inner port slides down to the correct position for sealing off right at the top of the neck.
9. Collect PRP from the **outer port** with a syringe/Luer Lock (no needle). Collects from the bottom up; retrieving the most concentrated first. Two 5ml syringes are included, but you may wish to use your own. The final needle should be no smaller than 30 gauge.
 - The total yield will be 12-15ml. Gently swirl if you'd like to use everything homogenously or don't swirl if you'd like to aspirate PRP first and then PPP.
 - For a greater concentration tilt the hourglass towards you at 45 degrees with the outer port at 6 o'clock and aspirate the desired volume. 1ml will yield a 16x concentration, 3ml - 10x and 5ml - 7x; for example.
 - Generally, 9% of the platelets will remain in the red blood cell layer, 11% reside in the buffy coat and 80% above the buffy coat.
 - If the yield is reddish in color (not golden) open the top collar a tiny bit more and spin again.