

### 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 1/3 turns counterclockwise. 1 1/2 turns is too much.
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 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. Raise the red blood cells and buffy coat until the buffy coat has entered into the top chamber of the hourglass with a firm grip on the tube and **slowly** turning the bottom collar clockwise - red blood cells will be in the neck and in the bottom chamber. The typical guide is for the top of the red blood cells to be just under the top of the neck of the tube (so that red blood cells do not enter the top chamber).  
See below for other options.
8. Once the buffy coat has been positioned into the top chamber 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 (which seals right at the top of the neck).
9. Collect PRP from the outer port with a syringe/Luer Lock (no needle). PRP and the buffy coat collect from the bottom up. The inner port is only used when isolating the buffy coat (so probably never). Two 5ml syringes are included, but you may wish to use your own. The final needle should be no smaller than 30 gauge.

**Option #1** - Aspirate the desired volume through the outer port. The total yield will be 12-15ml of Leukocyte-Rich PRP. 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.

**Option #2** - 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.

**Option #3** - For Monocyte-Rich PRP position half of the buffy coat in the neck. You will retrieve monocytes and lymphocytes with minimal neutrophils.

**Option #4** - Position entire buffy coat in the neck for Leukocyte Poor-PRP.

## Notes:

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.

Consider not using the buffy coat if it contains red blood cells, but (of course) use it if free of red blood cell contamination. You might allow a buffy coat with red blood cell contamination to stand for a few minutes to let the red blood cells settle out of the buffy coat into the red blood cell layer.

The bottom 3ml of PRP (in the CAREprp System) will contain the majority of platelets, but PPP is still a worthwhile layer with many useful components.

If red blood cells remain in the upper chamber please recheck that your top collar is open  $1 \frac{1}{3}$  times (by closing it and opening  $1 \frac{1}{3}$  times) and spin again to obtain optimal separation.