

Quick Start Guide

Companion Regenerative Therapies Companion Animal Health®



Companion Animal Health®
101 Lukens Drive, Suite A
New Castle, DE 19720

Phone 302.709.0408
Toll Free 877.627.3858
Fax 302.709.0409

Processing Whole Blood Samples for Platelet Rich Plasma (PRP) Isolation using the CRT PurePRP® Protocol

The Companion Regenerative Therapies PurePRP®II kit is designed to provide your clinic with the materials needed to efficiently process Whole Blood into Platelet Rich Plasma for therapeutic applications.

Included in the PurePRP® kit is:

- 6- Panel Labels (with Part and Lot numbers for records)
- 1- 60 mL syringe
- 2- 30 mL syringe
- 1- 10 ml syringe
- 1- Butterfly catheter
- 1- Surgical drape
- 10 mL ACD-A Anticoagulant
- 1- 60 mL Primary Concentrating Device
- 1-30 mL Secondary Concentrating Device
- (Counterbalance device and gram scale included with original CRT system kit)

Instructions for Use

Guidelines for Anticoagulant and Blood Draw Volumes

Size of Patient (in pounds)	Volume of ACDA (mL)	Volume of Whole Blood (mL)	Total Volume (Whole Blood + ACDA) (mL)	Final Volume of PurePRP® (mL)
<20	2.5	12.5	15	1
20-45	5	25	30	2
>45	10	50	60	4

***Please note:** The purpose of this table is to provide general guidelines for blood draw volumes and the correlating anticoagulant volume according to patient size. This table is not conclusive and all procedures should be performed under the consultation and direction of a licensed veterinarian.

Step 1: Prime the 60 ml Syringe for Blood Draw

Fill and prime the 60 ml syringe intended for the blood draw with the anticoagulant citrate dextrose solution formula A (ACD-A) (suggested volumes for patient size shown in the table above). Prime the 19g winged infusion set with ACD-A.

Step 2: Blood Draw

Draw from the jugular vein, filling the syringe to the pre-determined volume marker (“Total Volume” shown above) and invert the syringe several times to mix blood with anticoagulant.

Step 3: First Spin

Attach the syringe to the primary concentrating device and fill with collected blood by slowly pushing down the plunger. Once all blood has been transferred, detach the syringe from concentrating device. Using the gram scale, weigh the concentrating device and fill the counterbalance concentrating device until a corresponding weight is reached (within 2 gram difference). Place the concentrating devices in the centrifuge buckets at opposite sides of the rotor.

Set the centrifuge for 1 minute at 3600 revolutions per minute and press “Start”.

Step 4: Second Spin

Once the first spin cycle has finished (signaled by the opening of centrifuge lid), remove the concentrating device carefully from the centrifuge. Be careful not to shake or drop the device as this will re-suspend the cell layers which would require another 1 minute spin. Attach a 30 mL syringe and aspirate the platelet plasma suspension by pulling back on the plunger until red blood cells are seen at the top of the line. Detach the syringe from the first concentrating device and attach to the secondary concentrating device and fill slowly with plasma platelet suspension. Using the gram scale, weigh the concentrating device and fill the counterbalance concentrating device until a corresponding weight is reached (within 2 gram difference). Install 30 mL device inserts into centrifuge buckets and place the concentrating devices in the centrifuge buckets at opposite sides of the rotor. **Set the centrifuge for 5 minutes at 3800 revolutions per minute and press “Start”.**

Step 5: Final PRP Product

Once the final spin has concluded, remove the concentrating device carefully from the centrifuge. Be careful not to shake or drop the device as this will re-suspend the platelets which would require another 5 minute spin. Attach a new 30 mL syringe and slowly draw platelet poor plasma until plasma volume reaches the 4 mL mark on the device. Attach the 10 mL syringe and gently swirl the device until cells are re-suspend, indicated by a uniform color and minimal cell residue on the bottom of the device. Tilt the device to immerse the aspirating pipe and draw back on the syringe until all PurePRP® is collected. Remove the syringe from the concentrating device and place syringe cap on the end of the syringe until it's ready for use.

Your 4 mL of PurePRP® is ready to inject!

Processing Bone Marrow Aspirate for BMAC Stem Cell Isolation use the CRT PureBMC® Protocol

The Companion Regenerative Therapies PureBMC® kit is designed to provide your clinic with the materials needed to efficiently process Bone Marrow Aspirate Concentrate for therapeutic applications.

Included in the PureBMC® kit is:

- 6- Panel Labels (with Part and Lot numbers for records)
- 1- 60 mL syringe with 270-micron Bone Marrow Filter
- 1- Bone Marrow Aspiration Needle (11 Ga x 110 mm) with 60 mL Vaclock Syringe
- 2- 60 mL syringe
- 1- 10 mL syringe
- 1- 3 mL syringe
- 1- Surgical drape
- 5- Syringe caps
- 1- 60 mL Primary Concentrating Device
- 1- 30 mL Secondary Concentrating Device
- (Counterbalance device and gram scale included with original CRT system kit)
- 30 mL Heparin (packaged separately)

Instructions for Use

Step 1: Prime syringes and bone marrow filter with Heparin

Step 1. A: Using an 18g needle, fill and prime the 60 mL syringe (packaged with the 270-micron filter) with 5 mL of Heparin. Ensure complete coverage of the syringe with Heparin by pulling back on the plunger and inverting the syringe 2-3 times. Remove air from the syringe, then attach to the BMC filter at the “out” port and expel the Heparin into the filter. Keep the syringe attached to the filter and set aside for later processing steps.

Step 1. B: Attach the Vaclock syringe to 18g needle (still in Heparin container) and draw 10-15 mL of Heparin into the syringe. Ensure complete coverage of the syringe with Heparin by pulling back on the plunger and inverting the syringe 2-3 times. Attach the bone marrow aspiration needle to the 60 mL Vaclock syringe (with the plastic outer cover still on). With the needle pointed upwards, prime the bone marrow needle by pushing the plunger and expelling Heparin until 5 mL of heparin is left in the syringe. Detach the syringe and re-insert stylet for bone marrow needle placement.

Step 2: Collect the Bone Marrow

Remove the bone marrow aspiration needle cover and insert the needle into the proximal femur. Attach the 60 mL Vaclock syringe and draw 25 mL of bone marrow aspirate, filling the syringe to 30 mL. Ensure thorough mixture of Heparin and bone marrow aspirate during extraction through gentle rocking of the syringe. Detach the syringe and remove the bone marrow aspiration needle.

Step 3: Filter the Bone Marrow Aspirate

Remove the red cap from the “in” port of the filter and attach the Vaclock syringe containing bone marrow aspirate (the 60 mL syringe from Step 1.A should still be attached to the “out” port of the filter). To filter the bone marrow aspirate, firmly press down on the plunger of the Vaclock syringe while simultaneously pulling down the plunger of the receiving syringe*. Once all bone marrow has been filtered, disconnect the receiving syringe from the filter.

***Note:** It is normal for the bone marrow filter to become clogged with matter/bone fragments. Avoid excessive pushing/pulling pressure by slowly drawing the aspirate through the filter. Excessive force may cause the filter to fail.

Step 3: First Spin

Attach the 60 mL syringe with filtered bone marrow aspirate to the 60 mL concentrating device and transfer the sample by gently pressing down on the syringe plunger. Once all aspirate has been transferred, detach the syringe. Using the gram scale, weigh the concentrating device and fill the counterbalance concentrating device until a corresponding weight is reached (within 2-gram difference). Place the concentrating devices in the centrifuge buckets at opposite sides of the rotor.

Set the centrifuge for 1 minute at 4200 revolutions per minute.

Step 4: Second Spin

Once the first spin has concluded (signaled by the opening of centrifuge lid), remove the concentrating device carefully from the centrifuge. Be careful not to shake or drop the device as this will re-suspend the cell layers and would require another 1-minute spin. Attach the 60 mL syringe to the concentrating device. Aspirate the plasma by pulling back on the plunger until the bone marrow concentrate is seen in the top of the line. Remove this syringe and set aside for future processing. Attach the 3 mL syringe and aspirate 1 mL of bone marrow aspirate RBC layer. Remove the 3 mL syringe from the concentrating device and transfer both the BMA RBC layer (3 mL device- transfer this first) and the BMA plasma (60 mL syringe- transfer this second) into the 30 mL concentrating device. Using the gram scale, weigh the concentrating device and fill the counterbalance concentrating device until a corresponding weight is reached (within 2-gram difference). Place the concentrating devices into the centrifuge buckets at opposite sides of the rotor, using the 30 mL adaptors to secure them into the centrifuge buckets.

Set the centrifuge for 5 minutes at 4200 revolutions per minute.

Step 5: Collect BMC Pure

Once the second spin has concluded, carefully remove the concentrating device. With the 30 mL syringe, remove the BMA plasma until 4 mL of volume is remaining. Attach the 10 mL syringe and gently swirl the device until cells are re-suspend, indicated by a uniform color and minimal cell residue on the bottom of the device. Tilt the device to immerse the aspirating pipe and draw back on the syringe until all PureBMC® is collected. Remove the syringe from the concentrating device and place a syringe cap on the end of the syringe until it's ready for use.

Your 4 mL of PureBMC® is ready to inject!

CompanionRegenerativeTherapies.com



Companion Animal Health®
101 Lukens Drive, Suite A
New Castle, DE 19720

Phone 302.709.0408
Toll Free 877.627.3858
Fax 302.709.0409

RSR 000452 CRT Quick Start Guide