

Protocol step by step to obtaining Growth Factors- from Platelet Rich Plasma

Safety regulations and important considerations

"Always work within the laminar flow hood to prevent contamination.

"Clean laminar flow hood periodically during the procedure to maintain an aseptic environment.

"Be sure to collect blood samples prior to extraction of adipose tissue or bone marrow in case you are performing a procedure with stem cells.

»Spray regularly with disinfectant curtains laminar flow hood to maintain sterility.

"Beware of spraying tubes with disinfectant solution before placing them in the laminar flow hood.

"Do not let the spinal needle into contact with other elements to maintain sterility.

"Keep close a deposit of waste for easy removal.



Supplies

"[6] 8.5 ml tubes with anticoagulant

"[1] spinal needle 18 G

"[1] 10 cc syringe

"[2] alcohol wipes"[1] Latex Tourniquet

"[1] butterfly needle Vacutainer

"[1] Cap for Syringes

Note: All these materials are included in the bag 1 and 2 Regenestem Gold Kit for the isolation of SVF.

Equipment

1. Centrifuge Swing Out Rotor,with capacity to accommodate up to 50 ml tubes
2. Laminar flow hood with HEPA filter
3. Photoactivator Adi-light



Isolation of growth factors rich plasma

Step 1: Using a 21 g butterfly needle perform venipuncture and extract 50 to 55 ml of peripheral blood in the 6 tubes of 8.5 ml citrated included in the kit.

Step 2: Place the tubes in the centrifuge, set for centrifuging the samples at 2700 rpm for 7 minutes.

Step 3: Use the spinal needle 18 g and 20 ml syringe to aspirate the layer of platelet-rich plasma (PRP) plus buffy coat

to obtain 9 to 10 ml of plasma rich. Discard the tubes with red blood cells and platelet poor plasma (PPP).

Paso 4: Add 0.5 ml of calcium chloride 10%. Cap the syringe and shake gently to homogenize the mixture of calcium and PRP.

Paso 5: Place on the laser Adilight for 10 minutes.

Step 6: Hold the syringe in a horizontal position until clot retraction, which can take up to 45 minutes.

Step 7: Once retracted the clot, put the syringe into the laminar flow hood in order to subsequently reconstitute (pellet) of cells obtained from adipose tissue.