

SCCPRR STANDARD PROTOCOL FOR COLLECTION AND STORAGE OF SERUM

Based on discussions with scientists at BD and at Bristol-Myers, it seems that plasma is better than serum for “discovery” proteomics. This is partly due to the variables involved in the blood clotting process used to make serum. These variables can lead to varying degrees of proteolysis, make the search for biomarkers more difficult. Also, by using plasma, there is less chance of removing a protein of interest. If large amounts of fibrinogen or albumin do present a problem, there are depletion kits available to deplete the plasma of these proteins, although if this is done, associated proteins may be removed as well.

However, many groups are still using serum, because it contains fewer proteins. The method below is adapted from the one developed by Paul Tempst (Sloan Kettering), with the addition of protease inhibitors as recommended in the work by Hulmes, et al. (2004) of Bristol Myers.

Note: because there is a slight chance of “backstreaming”, these home-made collection tubes should not be used on human subjects. BD is working on a serum collection tube pre-loaded with protease inhibitors -- these should be available sometime in 2005.

1. Please refer to the P100 package insert (attached) for venipuncture techniques.
2. Prepare protease inhibitor solution (Sigma #P-2714) by adding 2.2 mL sterile deionized water. (You will be using 300 µL PIC solution/tube).
3. Preload BD tiger-top SST tube(BD # 367988) with 300 µL/tube PIC cocktail, by taking up ~0.4 µL PIC solution into a tuberculin syringe (BD #309623), forcing out air, and injecting 300 µL through the cap of the vacutainer tube. Remove needle quickly to preserve vacuum.
4. Collect venous blood into the BD tube, filling tube to top (8.5 mL).
5. Invert the tube slowly 8-10 times to mix protease inhibitors and coagulant with the blood.
6. Allow the blood to clot by standing tubes vertically at room temperature (22°C) for 60 min.
7. Place tubes in wet ice for no longer than 2 hours before centrifuging.
8. Centrifuge at 1400-2000 RCF for 10 min at 4°C. (see BD package insert to convert rpm to RCF).*
9. Within 30 minutes of centrifugation, transfer the supernatant (serum) in 1-mL aliquots to pre-labeled Fisherbrand 4-mL self-standing cryovials (Fisher Scientific # 0566966).
10. Place aliquots immediately on dry ice.
11. Freeze aliquots at -80°C until used. (Avoid freeze-thaw cycles).

*Note: removal of microplatelets would require an additional centrifugation step at 16,000 g for 20 min.

