

STANDARD PROTOCOL FOR COLLECTION AND STORAGE OF PLASMA

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, which can make the search for biomarkers more difficult. Also, 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.

Working with scientists at Bristol-Myers, BD has designed a sterile blood collection tube that is pre-loaded with protease inhibitors, as well as a self-contained system for removing red blood cells and platelets. These tubes come in sets of 12, and need to be stored at 4°C until needed.

The method below is based on the method BD has developed for these tubes, with a few small changes in the centrifugation step, etc.

1. Please refer to the P100 package insert (attached) for venipuncture techniques.
2. The BD P100 tubes are to be stored at 4°C, and should be prechilled at 4°C (not frozen) until use.
3. A full 8.5 mL of blood should be collected. This will produce about 2.5-3 mL of plasma.
4. Immediately after collection, invert the tube 8-10 times to mix the protease inhibitors and anticoagulant with the blood sample.
5. Place tube in wet ice before centrifuging. (Centrifugation should be done within 30 minutes of collection).
6. Centrifuge at 2000-3000 RCF at 4°C for 15 min. (See BD P100 package insert for converting rpm to RCF). Do not exceed 3000 g, or 10,000 RCF.
7. Within 30 minutes of centrifugation, transfer the plasma in 1-mL aliquots to pre-labeled Fisherbrand 4-mL self-standing cryovials (Fisher Scientific # 0566966).
8. Place aliquots immediately on dry ice.
9. Freeze aliquots at –80°C until used. (Avoid freeze-thaw cycles).

To remove microplatelets, the plasma should be transferred to a different centrifuge tube, and centrifuged at 12,000 g at 4°C for 15 min.