BIO-RAD

Sample Preparation for Ascites Fluid

I. Introduction

The following procedure is recommended for the preparation of ascites fluid samples prior to application onto any HPLC or medium pressure column or cartridge. This procedure will yield a highly clarified ascites fluid sample, and will help extend the life of the column or cartridge.

II. Procedure

1. After collection of the ascites fluid is complete, clot the ascits fluid sample by adding $CaCl_2$ to a final concentraion of ~1 mM (this will allow conversion of any existing fibrinogen to fibrin).

   Note: The use of heparin in the initial collection of the ascites fluid is not recommended.

2. Allow the ascites fluid sample to sit at room temperature for ~2 hours.

3. Rim the tube containing the ascites fluid to detach the fibrin clot from the wall of the tube.

4. Store the ascites fluid sample overnight at 4° C.

5. Remove the contracted fibrin clot from the ascites fluid with a wooden applicator stick, or other appropriate device, and discard the clot. The ascites fluid may now be frozen, if desired.

6. Clarify the ascites fluid sample by centrifugation at a minimum of 10,000xg for 30 mintures at 4° C. Retain the supernatant and discard the pellet.

7. Centrifuge the clarified supernatant at 100,000xg for 60 minutes at 4° C, if possible.

8. Carefully remove the lipid layer from the top of the sample. Decant and retain the supernatant and discard any remaining pellet.

9. Dilute or dialyze the clarified ascites appropriately depending upon the column chemistry to be used.

10. Filter the ascites fluid through a 1.0 μm membrane filter just prior to injection onto the column or cartridge.

11. The ascites fluid sample is now ready for loading.

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