

Handling of serum and plasma

Field scientist comments

Protein profiling of sera and plasma using SELDI

What constituents of blood do you want to look at? The answer to this question will determine how the blood should be processed – serum or plasma. The difference between serum and plasma is the removal of fibrinogen and clotting factors during the process of coagulation.

1) Plasma - If you want to see clotting factors and wound healing proteins/peptides you have to use an anti coagulant. Neither heparin nor EDTA is a problem in SELDI analysis so either can be used. The blood can then be centrifuged to remove the red blood cells (RBCs)

2) Serum - If you want to look at circulating proteins then clotting is a good way of removing RBCs and simplifying the blood. Serum is usually the better choice for profiling if you're not interested in profiling clotting factors or fibrinogen.

Storage of sera and plasma

The RBCs must be removed before storage of blood samples. Serum and plasma should be flash frozen in liquid nitrogen and stored at -20°C for the short term or -70°C for the long term. It is best to avoid freeze-thaw cycles, so aliquot samples prior to freezing. Most importantly, samples to be compared should have the same sample handling history whenever possible.

Further processing of sera and plasma

Hemoglobin removal

There should be limited red blood cell lysis with these samples. However, due to handling, samples are often “reddish” showing that they have suffered significant red cell lysis, leading to the presence of hemoglobin in the samples. Hemoglobin will dominate further SELDI analysis and it needs to be removed if the sample is red in color.

Hemoglobin has been found to have an affinity for Nickel and Cobalt. Therefore, Ni beads (which are commonly used for His tagged protein purification), or Co beads, can be used to deplete hemoglobin in serum samples

- Wash the Ni beads with PBS/0.3M NaCl,
- To 100µl lysate (red) in a 1.5ml microfuge tube, add 100µl 50% Ni beads suspension (~50µl beads in 50µl PBS/0.3M NaCl)
- Subject to 4 degree rotation for 20 min.

Following the incubation the lysate will be clear and the beads should have turned red, indicating that the hemoglobin has been depleted. The beads are then removed from the sample by centrifugation.

Although this method will deplete hemoglobin, it may also deplete other proteins in your sample therefore, it is recommended that care be taken on preparing your samples to minimize red blood cell lysis and concomitant hemoglobin contamination.

Delipidation

A problem with HUMAN serum can be the presence of large amounts of lipids, chylomicrons, free fatty acids and lipid carrying proteins. **If you are considering delipidating your sample, please contact your local Field Scientist for guidance on the latest methods.**

Some lipids can be removed by placing the serum on ice, then microfuging the sample at 4°C. This will isolate most of the lipid in an upper layer. This approach can be inefficient and variable, but helpful nonetheless.

There is a chromatographic resin for removing lipids called lipidx. Alternatively, lipids can be removed by extraction with ether/methanol or CHCl₃/methanol. Avoid using solvents like isoamylalcohol in the delipidation as they may extract peptides from the sera.