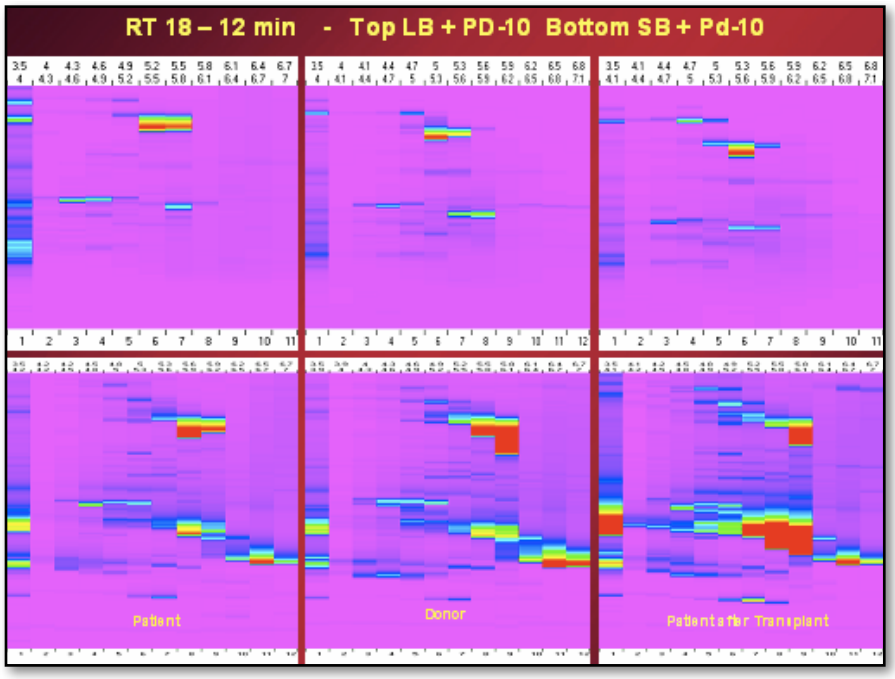
ProteoSep
TechNote 1

The "TCEP" Effect on Serum Sample Analysis



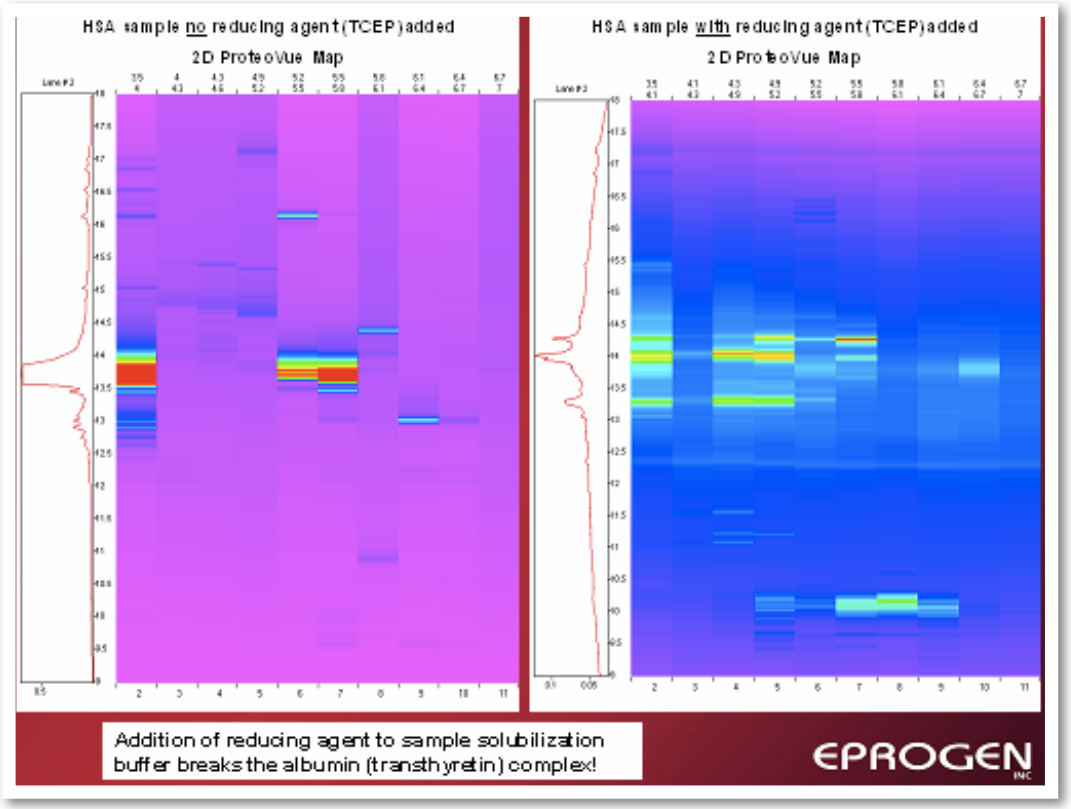
The analysis of serum samples using the ProteoSep chemistries has been shown to be markedly affected by sample solubilization procedure.

Conclusion: TCEP as a reducing agent should not be used when analyzing samples such as plasma, serum, CSF, amniotic fluid, or other high albumin containing samples. Start Buffer is sufficient to solubilize these sample types.

The ProteoVue maps displayed above overlay the results obtained for a set of three different patient serum samples treated by 2 different methods. The top three ProteoVue maps shows the results for PF2D runs where 300 uL of patient serum was dissolved in 2.2 mL of standard lysis buffer (LB) containing TCEP as a reducing agent followed by the standard PD-10 exchange protocol. The bottom three ProteoVue images are for the same three serum samples but this time the 300 uL was dissolved in 2.2 mL of Start Buffer (SB) followed by PD-10 exchange.

It is clearly evident that Albumin, in particular, as well as other components of serum are significantly altered by the solubilization protocol used. It was also observed that the CF column undergoes an irreversible protein capacity reduction after only a single run using the LB solubilization protocol.

Follow-up experiments using of HSA confirmed that TCEP, the reducing agent in the LB, is the source of the overall performance difference affecting the ProteoSep CF step in the 2D separation. The ProteoVue maps for 5 mg of HSA solubilized using LB with TCEP (Right Image) and LB without TCEP (Left Image) are highlighted below. Note: The CF column used on the left was run 3 additional times with 5 mg injections with no observed loss in performance whereas the CF column used on the right showed significant capacity loss after only one run.



Human serum albumin (HSA) contains 17 -X–S-S–X- bonds and is the mixture of human mercaptalbumin (HMA, reduced form) and human non-mercaptalbumin (HNA, oxidized form). [K Oettl and RE Stauber; British Journal of Pharmacology (2007) 151, 580–590].

TCEP is used to break protein X–S-S–X bonds for improved protein solubility. The observed irreversible capacity reduction after 2D runs with TCEP indicates a reaction with the CF ion-exchange sites, or the X–S-S–X bonds catalytically reform during the pH gradient causing the HSA protein to become entangled in the pores of the CF support. In either case, the ion-exchange capacity of the CF column is severely compromised and most all proteins are eluted in the basic wash fractions in subsequent ProteoSep runs. The addition of Iodoacetamide to cap the Sulfhydryl [X–S-H] groups formed by TCEP reduction was not successful in eliminating the "TCEP effect" during CF. This same effect was clearly observed when analyzing Amniotic Fluid samples as well (see Tech Note #2).