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PGPT™ & PGST™ – kit for Plasma and Serum Proteomics tube to Prevent Ex-vivo Protease Activity

Plasma and serum proteomics is of interest because of the potential presence of biomarkers for disease detection and prognosis. Sample preparation is a critical step in biomarker research and extreme care is needed to avoid or reduce pre-analytical and ex-vivo changes including enzymatic and non-enzymatic protein breakdown. Here we present the Proteomics Grade Serum / Plasma Tube (PGST/PGPT) that streamlines this critical step by inhibiting protease inhibitors at the point of blood collection thereby eliminating the need to transport samples frozen or in a hurry to a laboratory for analysis. The instant stabilization of plasma/serum proteins during sample collection improves reproducibility, data quality and confidence.

The central objective of many proteomics experiments is to identify biomarkers that can play a role in disease detection, progression, prognosis, mechanism and treatment[1,2]. Serum and plasma are of particular interest in biomarker discovery research because they have the potential to reveal biologically, clinically and mechanistically relevant proteomic signatures. Mass spectrometry plays a major role in proteomics and biomarker research[3] and it is presently one of the best technologies available for qualitative and quantitative interrogation of serum and plasma. The analysis of plasma and serum using the best proteomics technologies will however produce inaccurate data or data that is difficult to interpret if excessive and unpredictable pre-analytical changes occurred between sample collection and sample analysis.

A major cause of pre-analytical change in plasma and serum is the intrinsic protease activity[4]. Typically the blood collection step is done in the clinical setting and mostly away from the laboratory where the proteomics experiment will be performed. Thus the extent of the change in the proteome will vary and depend on the time interval between sample collection and stabilization. Inappropriate handling of plasma and serum is detrimental to already low abundant biomarkers, precluding or complicating their identification. To prevent pre-analytical changes it is necessary to keep the collected blood frozen soon after collection. But freezing and thawing may compound the problem by resulting in a breakdown of cells leading to further release of proteases. ITSI-Biosciences have developed Proteomics Grade Serum / Plasma Tube (PGST/PGPT) that stabilizes plasma and serum proteins at the point of collection to prevent the protease induced pre-analytical changes that may skew the proteomics data. The kit also protects plasma and serum proteins from protease activity when frozen samples are thawed. Unless the plasma and serum samples are handled appropriately to prevent pre-analytical changes the identified candidate biomarker may just be a reflection of the effect of the blood collection, handling and/or storage protocol on the biological or clinical sample.

Materials & Methods:

Blood sample was collected from a fully informed and consented 45 years old donor using either a standard vacutainer tube for plasma (VT, Becton Dickinson, Franklin Lakes, NJ) or PGPT manufactured by ITSI-Biosciences, LLC (Johnstown, PA). Plasma was isolated from the tubes after approximately 24 hours of storage at room temperature according to standard protocols. The isolated plasma was stored at -80^{o}C until analyzed. Subsequently, samples were thawed on ice and analyzed as described below.

The Agilent Bioanalyzer 2100 was used to analyze the VT and PGPT plasma samples to check for potential differences in the protein profiles. Briefly, 2 µl of plasma was diluted with 2 µl of 1X PBS prior to analysis with the Agilent Bioanalyzer 2100 using the Protein 80 chip. Agilent protein 80 sample buffer was supplemented with 3.5% v/v of mercaptoethanol and 2 µl was added to each sample aliquot. The samples were then heated at 95°C for 5 min. A tube with 6 µl of Agilent protein 80 molecular weight markers was also heated for 5 min. The denatured and reduced samples and the molecular weight marker were diluted with the addition of 84 µl of milli-Q water. The Agilent protein 80 chip was primed according to manufacturer's instructions and 6 µl of diluted sample

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was loaded into the sample wells on the chip. The chip was then run on the Bioanalyzer using the protein 80 assay program and the data collected.

Results:

The Agilent Bioanalyzer allowed the qualification and quantitation of the resolved protein bands in the plasma samples. Figure 1 shows the profile and peak area for selected plasma proteins in VT-plasma and PGPT-plasma.

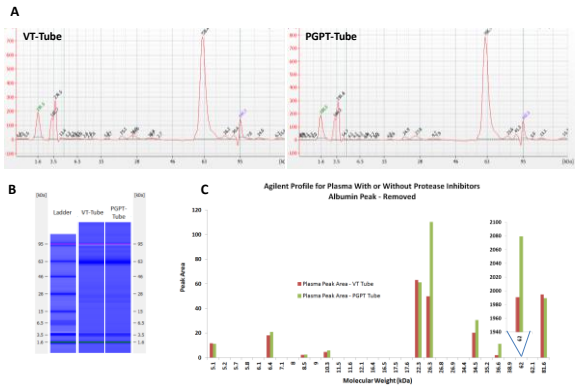

Figure 1: **Protein Profile analysis using Agilent Bioanalyzer for comparing VT and PGPT tubes.** Panel A shows the Agilent Bioanalyzer Electropherograms profile for VT and PGPT-Tube. The gel view of the same is shown in B. Panel C shows the plot of Peak Area vs. molecular weight (kDa). 70 % of the peaks in VT samples show a drop in peak area compared to peaks in PGPT. The greatest decrease was observed with the proteins at 26.3kDa and 62kDa (albumin peak).

Each peak represents proteins with similar molecular weights. For example the peak around 62 kDa represents albumin peak in VT and PGPT samples. The corresponding peak area represents the estimated amount of albumin present in each sample. Peak area was measured for peaks between 4.8 kDa and 90 kDa. Hence, if a protein is digested by the proteases to peptides then it may be outside the detection limit of the Agilent Bioanalyzer. Using all the well-resolved bands in the higher molecular weight region as an index, we determined that there was an overall decrease of ~36% in the peak area representing full length plasma proteins in VT compared to similar proteins in PGPT. Furthermore, we selected a total of 10 clearly resolved bands precisely determine the difference in the peak areas in the VT and PGPT samples. Among them, 7 (70 %) in VT-plasma showed a drop in peak area compared to PGPT-plasma. Since the peak area is a direct reflection of the protein concentration, a decrease in the peak area of a protein in VT-plasma that does not also occur in PGPT-plasma suggests that a breakdown occurred in VT-plasma. Specifically, the concentration of albumin the most abundant protein in plasma was calculated as 0.97 µg/µl in PGPT and 0.90 µg/µl in VT. This represents ~7% decrease in albumin concentration in VT compared to PGPT.

The results obtained underscore the significance of stabilizing plasma and serum proteins at the point of collection. Proteases present intrinsically in plasma and serum are non-specific and will result in loss of proteins in a random fashion resulting in variation in the downstream data collection step. This phenomenon will affect both the number of proteins identified and protein sequence coverage. Inhibition of protease activity at the point of sample collection will reduce predictable protease induced variations and will result in a more reliable biomarker analysis. It is worth mentioning that the concentration changes in full length proteins detected with the Agilent Bioanalyzer will not be apparent if the plasma samples were analyzed by mass spectrometry via shotgun proteomics. This is because Shotgun proteomics, also termed “bottom up proteomics” relies on first digesting all proteins into peptides prior to sequencing of the peptides by mass spectrometry.

In conclusion, ITSI – Bioscience's Proteomics Grade Plasma Tube (PGPT) was found to be effective in reducing pre-analytical changes attributable to protease activity. PGPT tubes contain a cocktail of protease inhibitors with specificity for the inhibition of serine, cysteine, aspartic proteases and aminopeptidases. The plasma sample from PGPT has been analyzed by Mass spectrometry, SDS-PAGE, 2D-DIGE and Western blotting without the need to remove the inhibitors. Elimination/reduction of sample-to-sample variation will increase confidence in the result obtained since the difference in protein abundance detected between samples will not be attributable to ex-vivo protease activity. PGPT also saves time by eliminating the need to pre-mix or transfer plasma from regular collection tubes.

References:

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