

# Multiplexed Detection of Tumor Biomarker Proteins

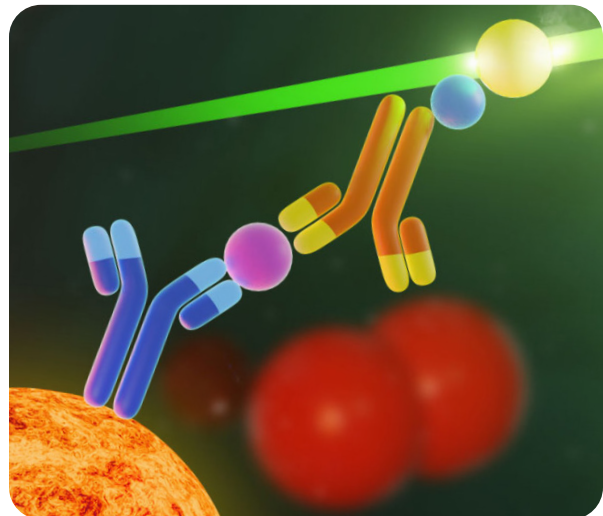
## Using Luminex Assays and Streck Plasma

### Introduction

**Luminex® High Performance assays** from R&D Systems™, a Bio-Techne brand, offer a powerful tool for the multiplexed detection of high-importance biomarkers while providing high sensitivity and industry-leading precision and reproducibility. With the newly released Human Tumor Biomarker Performance Panel, a 28-plex immunoassay measuring soluble tumor biomarkers, these Luminex assays for the first time have been validated for use with plasma samples collected with **Streck® Cell-Free DNA BCT®** or **Streck Protein Plus BCT™**. Leveraging advanced Luminex xMAP® technology, this powerful solution includes a comprehensive array of biomarkers pivotal in understanding tumor biology, progression, and response to therapy. By incorporating a wide spectrum of tumor markers, including those associated with liver cancer, ovarian cancer, breast cancer, pancreatic cancer, neuroendocrine tumors, and thyroid cancer, it supports comprehensive profiling and fosters a deeper understanding of tumorigenesis and metastasis. Each biomarker has been validated to ensure optimal performance. The combination of these high-quality assays and sample types will improve the integration of genomic and proteomic tools for multicancer early detection research and diagnostic development.

Streck Cell-Free DNA BCT contains preservatives that maintain cell-free DNA and limit the release of genomic DNA, minimizing the degradation of circulating tumor cells that are crucial to many downstream research and development applications. The Streck Protein Plus BCT contain preservatives designed to minimize *ex vivo* hemolysis and platelet activation in the collected sample, limiting interference from proteins released post-draw that

can add error to the true abundance of the circulating analytes of interest. The stabilizing effects in the Streck tubes allows for the collection of samples from multiple sites while utilizing a single site for sample processing and analysis. The added stability time means researchers can ship samples without worrying about lapses in cold chain storage, or about differences in sample handling at the collection sites.



### Methodology

The stability of 28 multiplexed analytes was evaluated using the Human Tumor Biomarker Performance Panel (Catalog # **FCSTM25**). Samples from 20 presumed healthy individuals were collected in EDTA, Heparin, Streck Cell Free DNA BCT, and Streck Protein Plus BCT. Plasma was isolated and processed per the manufacturer's guidelines after 0, 3, or 5 days post-draw at room temperature. Aliquots of processed samples were stored at -20 °C until time of evaluation.

The EDTA and Heparin samples were diluted 2-fold with assay diluent, while the Streck BCT and Protein Plus samples were diluted either 2-fold or 8-fold with assay diluent. Four sets of assays were performed on separate days. Reported concentrations were back-calculated for the appropriate dilution factor.

## Results

The Streck Cell Free DNA BCT and Protein Plus BCT demonstrated superior stability for the analytes in the Human Tumor Biomarker Panel compared to EDTA and heparin tubes. As shown in Figure 1, limited

analytes had sample stability observed across all tube types. Figure 2 highlights the consistent performance of multiple analytes in the Cell Free DNA and Protein Plus BCT tubes, while the EDTA and heparin tubes exhibited variability at days 3 and 5.

Additionally, Figure 3 shows that both Streck tubes performed exceptionally well with IL-8, which forms heterodimers with platelet factor 4 (PF4), a protein released at high concentrations from platelets. IL-8 concentrations were notably elevated in EDTA and heparin plasma samples at days 3 and 5.

**FIGURE // 01**  
Analyte Stability

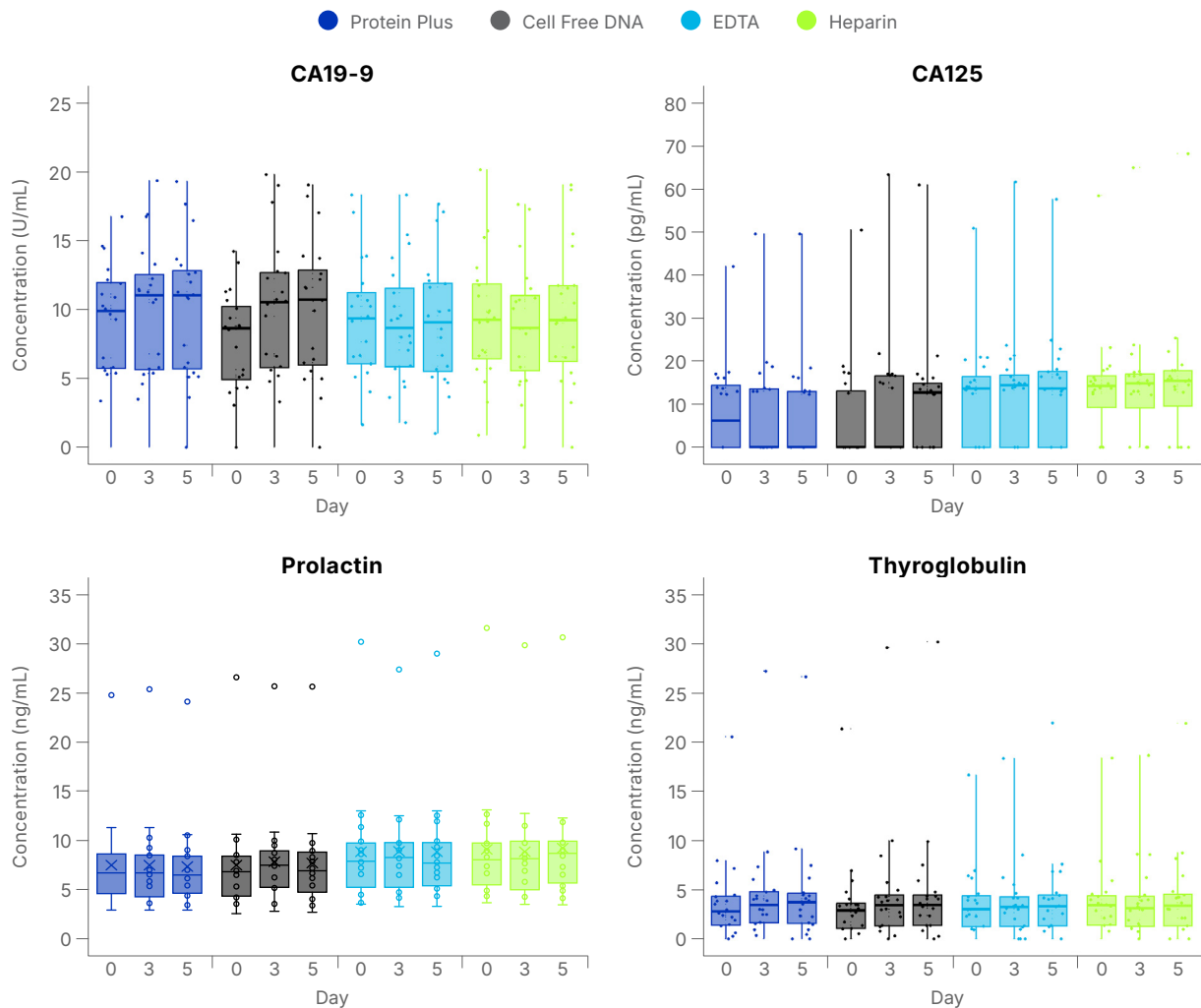


Figure 01. CA19-9, CA125, Prolactin, and Thyroglobulin sample levels remain consistent across all tubes and days.

**FIGURE // 02**

**Performance in Protein Plus BCT Tubes**

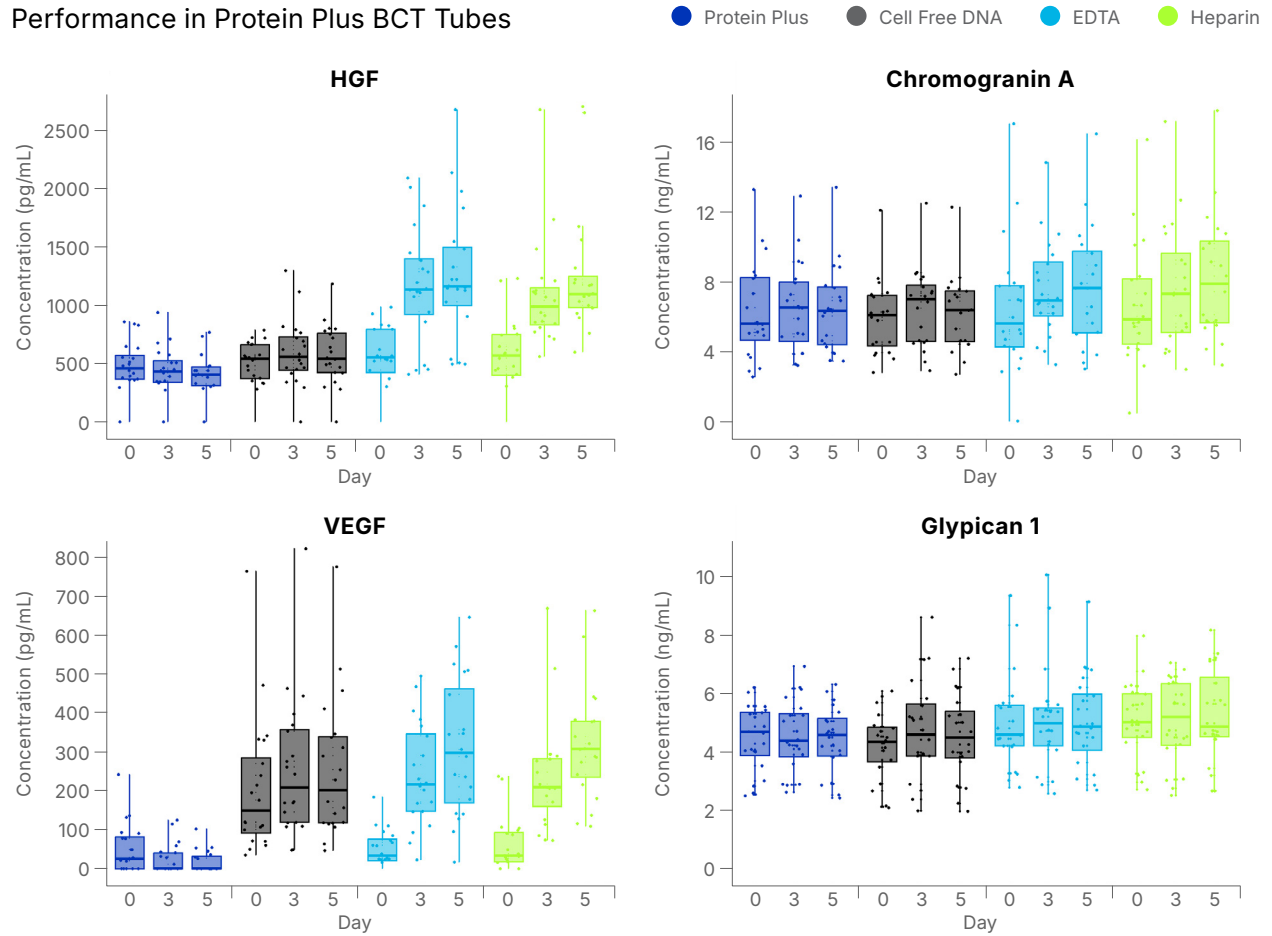


Figure 02. Sample values remained stable in Streck Cell Free DNA BCT and Protein Plus tubes while sample values increased over time in EDTA and heparin.

**FIGURE // 03**

**Stability Over Time**

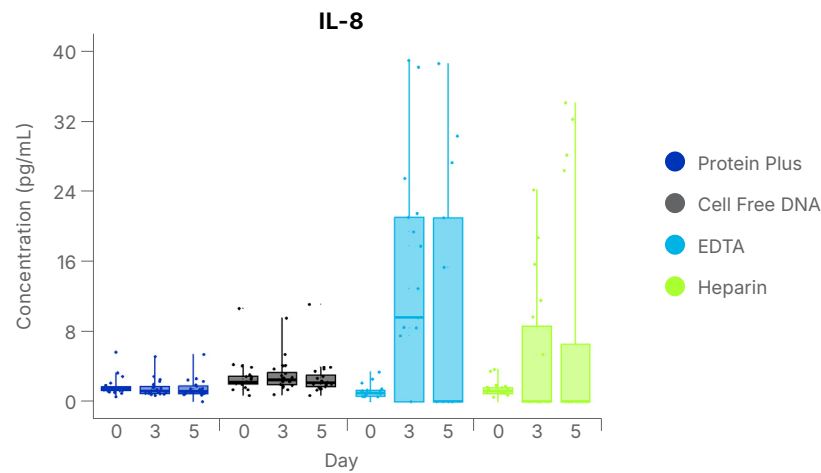


Figure 03. IL-8 is known to form heterodimers with PF4. In both Streck Cell Free DNA BCT and Protein Plus tubes levels remained stable over time while both EDTA and heparin IL-8 levels continued to increase over time.

## Summary

The combination of Streck plasma tubes and Bio-Techne's Human Tumor Biomarker Performance Panel are a winning combination for stability and sensitivity in the discovery and development of soluble tumor biomarkers associated with cancer. The Streck tubes stabilized most of these important tumor biomarker analytes when stored at room temperature prior to processing and evaluation. The Protein Plus tubes were the best at stabilizing analytes with known stability issues related to platelet activation and release (including CD40 ligand, IL-8, and MIF).

Streck Cell-Free DNA BCT outperformed Protein Plus BCT for ENO-2 and MIF stability. The tube type and collection procedure can have an impact on the stability of some analytes. It is important to test analyte stability early in studies with large cohorts. Any large study should involve the validation of possible sample handling differences across sites and could follow a similar study plan to what is presented here.

## Conclusion

The integration of the Human Tumor Biomarker Luminex Performance Panel with Streck® Cell-Free DNA BCT and Protein Plus BCT offers a robust solution for multiplexed detection of tumor biomarkers, ensuring high stability and accuracy in plasma samples even under varied conditions. Our findings demonstrate that these tubes effectively preserve the integrity of critical analytes, with the cfDNA BCT showing superior stability for certain platelet-sensitive markers like MIF and IL-8. Protein Plus BCT also excels in stabilizing analytes prone to degradation due to platelet activation. These results underscore the importance of selecting the appropriate sample collection and handling methods in large-scale cancer studies, as they directly impact the reliability of biomarker detection. This combined approach of advanced assay technology and optimized sample preservation stands to significantly enhance the precision and reproducibility of multicancer biomarker research and diagnostics.



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