

Addressing the Challenge of Pre-analytical Variables in Circulating Protein Biomarker Analysis for Liquid Biopsy

Rachel M. Miller, Ph.D., and Jing Li, Ph.D.
Streck LLC., La Vista, NE, United States



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INTRODUCTION

While liquid biopsy using plasma proteins is a promising avenue for early cancer detection, diagnosis, prognosis and disease monitoring, the clinical translation of plasma proteomics has been limited due to the complexity of the proteome and the impact of pre-analytical variation on its integrity. In recent years, advancements in plasma proteomic technologies, such as Olink®, have helped address the challenge of proteome complexity. However, challenges with pre-analytical variables have persisted. During blood collection and whole blood storage, *ex vivo* blood cell activation and lysis can occur, releasing proteins into the plasma and obscuring relevant *in vivo* abundances. This is especially true for cancer where many key biomarkers are expressed in white blood cells and platelets. To address these issues, Streck developed Protein Plus BCT™, which stabilizes blood cells and minimizes plasma proteome changes following prolonged whole blood storage. Here, we demonstrate the impact of prolonged whole blood storage on the plasma proteome of blood collected into conventional anticoagulants (EDTA, ACD-A) and highlight the functionality of the Protein Plus BCT in better maintaining draw time plasma proteome levels using Olink Proximity Extension Assay (PEA) technology.

Protein Plus BCT is for Research Use Only. Not for use in diagnostic procedures. Protein Plus BCT should only be used for research or the development of new assays.

METHODS

Blood was collected from five self-proclaimed healthy donors into EDTA, ACD-A and Protein Plus BCT. Plasma was isolated from whole blood at draw time (within 2 hours), or after 24 hours, 72 hours and 120 hours of ambient temperature storage using a double spin protocol (1800 xg for 15 min, 2800 xg for 15 min) and frozen at -80 °C until analysis. Plasma samples were sent to the Vanderbilt University Medical Center High-Throughput Biomarker Core Lab for analysis using the Olink Explore 384-plex Cardiometabolic panel. Resultant data was analyzed at Streck.

RESULTS

Plasma levels of biomarkers of interest are maintained by Protein Plus BCT.

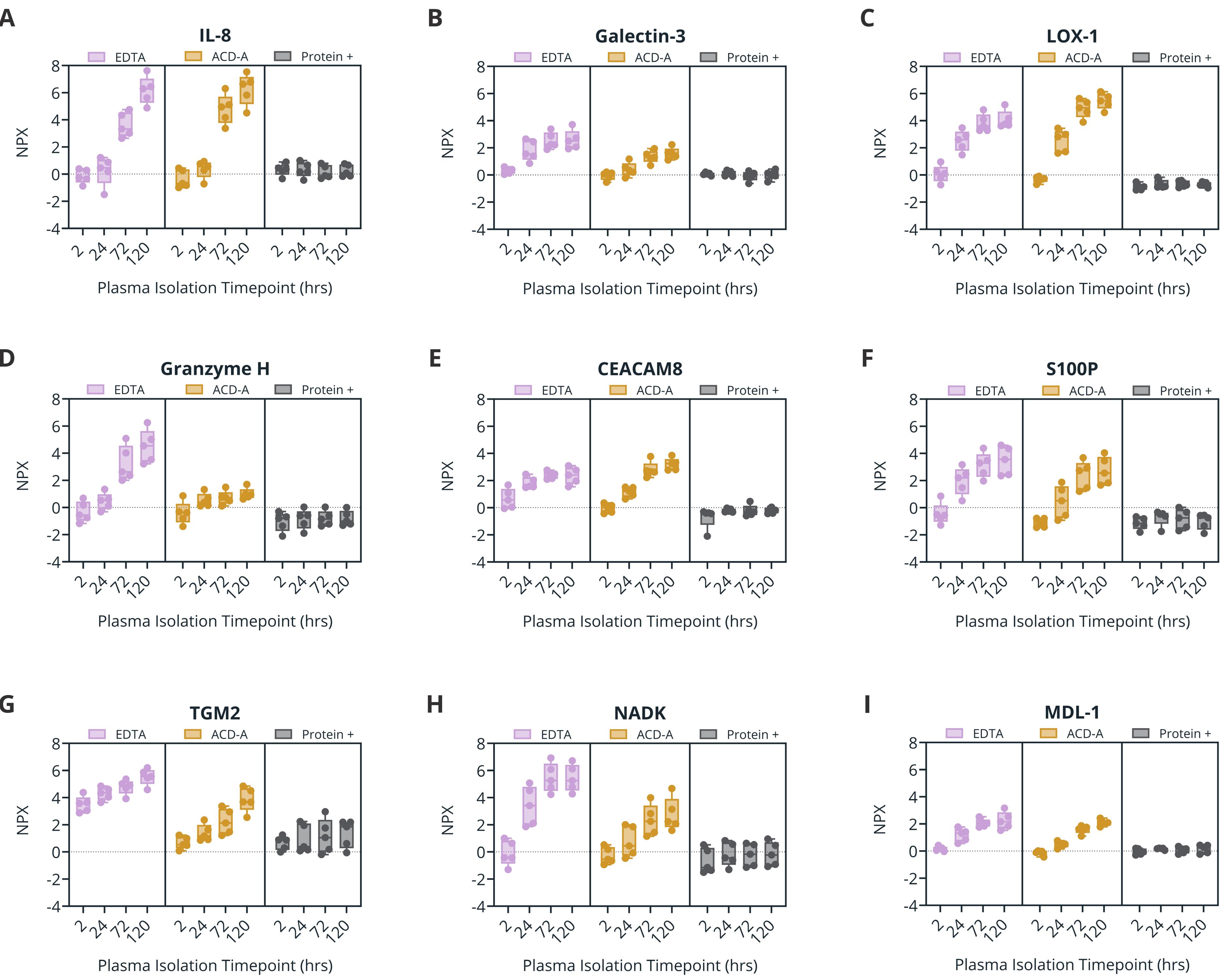


Figure 1. (A-I) Plasma levels, as represented by NPX (normalized protein expression unit reported on a Log₂ scale), of protein biomarkers from samples isolated from EDTA, ACD-A and Protein Plus BCT at draw time (2 hrs) and after 24, 72 and 120 hours of ambient temperature whole blood storage.

RESULTS (cont.)

Protein Plus BCT limits changes to the plasma proteome during whole blood storage.

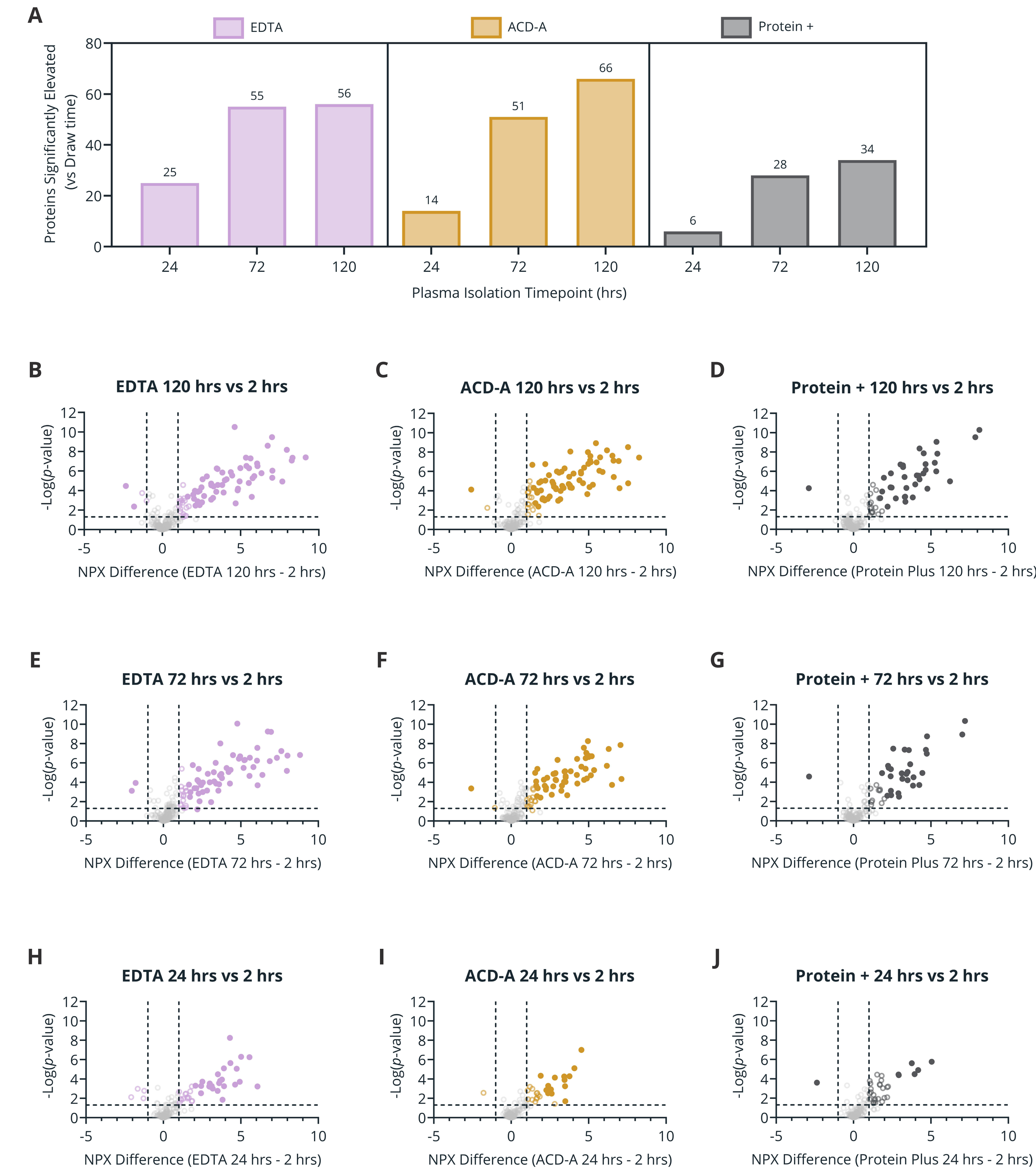


Figure 2. (A) Number of proteins significantly elevated in abundance after 24, 72 or 120 hrs of whole blood storage in EDTA, ACD-A and Protein Plus BCT relative to draw time (2 hrs). **(B-J)** Differential analysis of plasma protein abundances between plasma isolated from blood drawn into EDTA, ACD-A or Protein Plus BCT at draw time (2 hrs) versus after 120 hrs **(B-D)**, 72 hrs **(E-G)** or 24 hrs **(H-J)** of storage at ambient temperature. **(H-J)**. Colored outlines of markers indicate an NPX Difference >1 or <-1 with a -Log(*p*-value) > 1.3 and filled markers indicate FDR ≤ 1%.



RESULTS (cont.)

Protein Plus BCT minimizes abundance changes in proteins associated with blood cell activation, movement and vesicles.

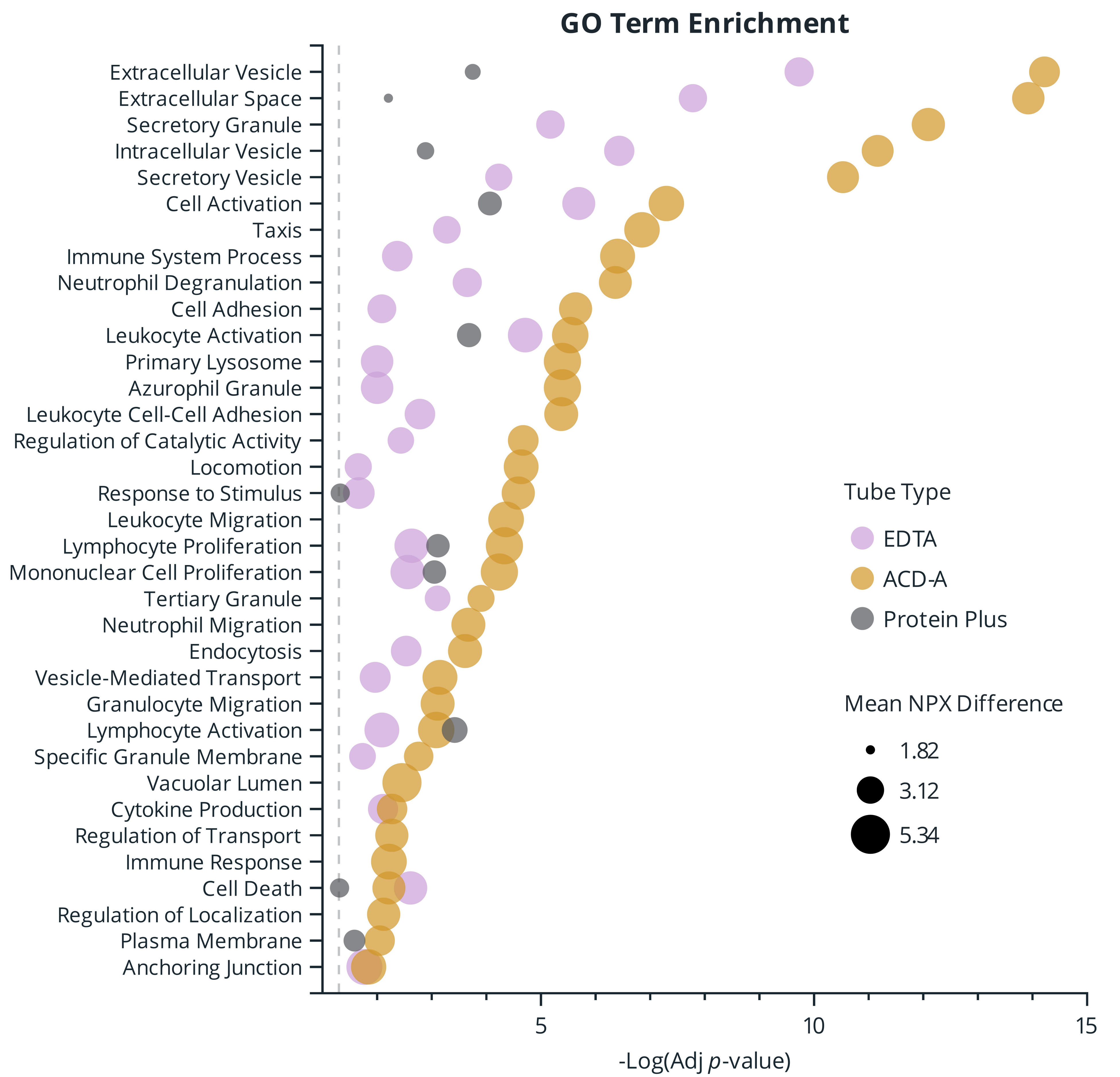


Figure 3. Gene Ontology (GO) enrichment analysis of the proteins with statistically significant changes in abundance after 120 hours of whole blood storage in EDTA, ACD-A and Protein Plus BCT. Of the 35 GO Terms enriched in conventional anticoagulants, samples collected into Protein Plus BCT display enrichment in only 11. For those terms where Protein Plus is enriched, the magnitude of change is less than observed in EDTA and ACD-A. Less overall enrichment and lower magnitude of changes demonstrate that Protein Plus BCT better maintains draw-time protein abundances. Dashed line indicates adj *p*-value of 1.3 (adjusted *p*-value of 0.05).

CONCLUSION

These data demonstrate that plasma isolated from Protein Plus BCT is compatible with Olink PEA technology. Further, Protein Plus BCT greatly limits the *ex vivo* activation, lysis and degradation of blood cells often seen in samples collected and stored in conventional anticoagulants (EDTA, ACD-A). By extending the storage of whole blood at ambient temperature without compromising the integrity of the sample or the proteomic results, Protein Plus BCT provides researchers and assay developers valuable flexibility in the storage and processing of their samples.



Contact BD@streck.com for more information about working with Streck!