

Running standard matrices

Analysis of Plasma and Serum

Study Design Considerations

Protein assays within Olink panels have been optimized for the dynamic range present in human plasma and serum. Descriptions of how [Target](#) (Ref 1) and [Explore](#) (Ref 2) platforms were validated have been described in detail. Results are reported as NPX™ units which are used to compare relative changes in protein abundance between study groups. Identification of true biological differences between study groups is facilitated by reducing technical variability to the fullest extent possible. This includes using the same collection procedure for each sample (e.g., either serum *or* EDTA-plasma *or* heparin-plasma *or* citrate-plasma), maintaining even storage conditions ($\leq -80^{\circ}\text{C}$), and keeping the same number of freeze/thaw cycles. Other potential confounding factors can result from blood components such as hemolysis products, bilirubin, and lipids, however these factors have been tested during our validation and shown not to affect the data unless at extreme levels (Ref 3). Please consult our white paper on pre-analytical variation for more information (Ref 4).

Within a particular study, all samples should be randomized across all plates, and it is best to use a balanced number of samples across the study groups. Replicates are not necessary since internal and external controls in the Olink system are used to control for technical variability (Ref 5). Samples are normalized by volume.

It is not possible to obtain plasma or serum from whole blood samples that have previously been frozen. Frozen whole blood can be analyzed by following Olink's guidelines for dried blood spots.

In addition to the standard sample preparation procedures described below, plasma from BD™ P800 blood collection, Cell-Free DNA BCT® Streck tubes, and serum from Tasso-SST® devices are compatible with Olink and can be analyzed using the standard dilution strategies for plasma and serum.

Recommendations for Sample Preparation

Serum

- Collect whole blood into serum collection test tubes
- Allow the blood to clot at room temperature, which usually takes 15-30 min
 - Note:* Red blood cells are more likely to lyse in samples left for more than 60 min at room temperature, and hemolysis can be a confounding factor in data analysis
- Centrifuge for 10 min at 1000-2000 x g at 4°C to remove the clot
- Immediately transfer the serum into a clean tube and mix
- Aliquot and store at -80°C or lower

Plasma

- Collect whole blood into commercially available anticoagulant-treated tubes (e.g., EDTA-, citrate- or heparin-treated), invert several times to ensure correct mixing
- Centrifuge for 10 min at 1000-2000 x g at 4°C to remove cells from the plasma
Note: Process as quickly as possible after collection (within 30 min); store tubes at 4°C if necessary
- Immediately transfer the plasma into a clean tube and mix
- Aliquot and store at -80°C or lower

Pre-Dilution Strategies

Target 96:

CAM	CRE	CVDII	CVDIII	DEV	IMO	INF	IRE	MET	NEU	NEX	ODA	ONCII	ONCIII
1:2025	1:1	1:1	1:100	1:100	1:1	1:1	1:1	1:10	1:1	1:1	1:1	1:1	1:1

Target 48:

1:1

Note: Dilutions are denoted as A:B, where A=number of sample units and B=total number of units after dilution, therefore 1:1 = undiluted or 'neat' sample.

References

Ref 1: Assarsson E, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One*. 2014; 9(4):e95192. DOI: 10.1371/journal.pone.0095192. [Link](#)

Ref 2: Wik L, et al. Proximity extension assay in combination with next-generation sequencing for high-throughput proteome-wide analysis. *Mol Cell Proteomics*. 2021; 20:100168. DOI: 10.1016/j.mcpro.2021.100168. [Link](#)

Ref 3: Further information can be found in Olink panel validation documents, including Target 96 Inflammation. [Link](#)

Ref 4: Olink white paper: Pre-analytical variation in protein biomarker research. [Link](#)

Ref 5: Olink white paper: Data normalization and standardization. [Link](#)

Publications using Olink

Wei TT, et al. Cannabinoid receptor 1 antagonist genistein attenuates marijuana-induced vascular inflammation. *Cell*. 2022; 185(10):1676-1693.e23. DOI: 10.1016/j.cell.2022.04.005. [Link](#)

Arunachalam PS, et al. Systems vaccinology of the BNT162b2 mRNA vaccine in humans. *Nature*. 2021; (7872):410-416. DOI: 10.1038/s41586-021-03791-x. [Link](#)

Narula S, et al. Plasma ACE2 and risk of death or cardiometabolic diseases: a case-cohort analysis. *Lancet*. 2020; 396(10256):968-976. DOI: 10.1016/S0140-6736(20)31964-4. [Link](#)

Please contact support@olink.com for further information on running standard matrices.

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