

Analysis of Interstitial Fluid

Study Design Considerations

Protein assays within Olink panels have been optimized for the dynamic range present in human plasma and serum. 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, keeping the same number of freeze/thaw cycles, and maintaining even storage conditions.

Within a study, all samples should be randomized across all plates and it is best to use a balanced number of samples across the study groups.

In addition to plasma and serum, strategies have been developed to analyze alternative types of samples. Interstitial fluid fills space between cells and it is distinct from blood plasma. It facilitates nutrient delivery to cells, intercellular communication, and removal of metabolic waste. Because interstitial fluid does not clot like blood, dermal sampling could offer a new approach for continuous and routine clinical monitoring and testing as well as a minimally invasive technique for investigative research. Internal tissues can be sampled by microdialysis: a catheter is inserted that contains a dialysis membrane and proteins are collected in circulating perfusate.

Samples are normalized by volume. To evaluate protein assays at risk for hook it is recommended to run a few samples from each study group at two additional dilutions. It is not necessary to include biological replicates or to add protease inhibitors. Technical replicates can be included for better estimation of CVs when using an alternative matrix.

Recommendations for Sample Preparation

Sample collection

- Interstitial fluid should be collected using best practice clinical guidelines.
- Freshly collected samples are stable for a short duration at room temperature but should be stored on ice or at 4°C if possible.

Note: You may choose to add protease inhibitors to the samples. Roche cOmplete™ Mini Protease Inhibitor Cocktail (#11836153001) is recommended. A 10X solution can be prepared by dissolving 1 tablet in 1 ml of distilled water or PBS, or a 7X stock in 1.5 ml. The stock solution can be stored at 4°C for ≤2 weeks or -20°C for ≤12 weeks. Use a 1X final concentration of inhibitor cocktail and avoid excess final concentrations (e.g., 2X or 3X)

- Samples should be centrifuged for 10 min at ≥500 x g to remove cells and insoluble material.
- Aliquots should be stored at -80°C.

Pre-Dilution Strategies

Target 96:

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

Publications using Olink

Dyhrfort P, et al. A dedicated 21-plex proximity extension assay panel for high-sensitivity protein biomarker detection using microdialysis in severe traumatic brain injury: The next step in precision medicine? *Neurotrauma Rep.* 2023; 4(1):25-40. DOI: 10.1089/neur.2022.0067. [Link](#)

Ekstrand J, Abrahamsson A, Lundberg P, Dabrosin C. Breast density and estradiol are associated with distinct different expression patterns of metabolic proteins in normal human breast tissue in vivo. *Front Oncol.* 2023; 13:1128318. DOI: 10.3389/fonc.2023.1128318. [Link](#)

Charrez B, Charwat V, Siemons BA, Goswami I, Sakolish C, Luo YS, Finsberg H, Edwards AG, Miller EW, Rusyn I, Healy KE. Heart muscle microphysiological system for cardiac liability prediction of repurposed COVID-19 therapeutics. *Front Pharmacol.* 2021; 12:684252. DOI: 10.3389/fphar.2021.684252. [Link](#)

Lindahl G, Abrahamsson A, Dabrosin C. Dietary flaxseed and tamoxifen affect the inflammatory microenvironment in vivo in normal human breast tissue of postmenopausal women. *Eur J Clin Nutr.* 2019; 73(9):1250-9. DOI: 10.1038/s41430-019-0396-y. [Link](#)

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