

Running alternative matrices

Analysis of Synovial 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. Synovial fluid is a thick liquid located between the joints that acts as a shock absorber and helps to alleviate friction. Proteomic analysis of synovial fluid can aid in the investigation of certain diseases such as arthritis, particularly septic or crystal-inducing arthritis.

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. Highly viscous biofluids can impede accurate pipetting, therefore dilution of synovial fluid is recommended.

Recommendations for Sample Preparation

Sample collection and preparation

- Synovial 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.
- Samples should be centrifuged for 10 min at $\geq 2000 \times g$ to remove cells and insoluble material.
- If necessary, samples can be diluted 1:4 with Olink diluent to reduce viscosity. All samples should be treated equally regardless of viscosity.
- It is not recommended to include samples which appear to have blood contamination.
- 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
1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4

Target 48:

1:4

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

Barbarroja N, et al. Characterization of the inflammatory proteome of synovial fluid from patients with psoriatic arthritis: Potential treatment targets. *Front Immunol.* 2023; 14:1133435. DOI: 10.3389/fimmu.2023.1133435. [Link](#)

Struglics A, Larsson S, Lohmander S, Swärd P. Technical performance of a proximity extension assay inflammation biomarker panel with synovial fluid. *Osteoarthr Cartil.* 2022; 100293. DOI: 10.1016/j.jocarto.2022.100293. [Link](#)

Aulin C, Larsson S, Vogl T, Roth J, Åkesson A, Swärd P, Heinbäck R, Erlandsson Harris H, Struglics A. The alarmins high mobility group box protein 1 and S100A8/A9 display different inflammatory profiles after acute knee injury. *Osteoarthr Cartil.* 2022; 30(9):1198-1209. DOI: 10.1016/j.joca.2022.06.009. [Link](#)

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