

Analysis of Cerebrospinal 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.

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.

In addition to plasma and serum, strategies have been developed to analyze alternative types of samples. Cerebrospinal fluid (CSF) is a clear, colorless fluid that surrounds the brain and is found within the spinal cord. CSF reflects nervous system pathology and can provide insight into neurological and infectious diseases. Normalization of CSF samples is based on sample 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. Technical replicates can also be included for better estimation of CVs when using an alternative matrix.

Study samples which appear to have blood contamination should be noted. It is not recommended to include contaminated CSF samples because: i) blood-derived proteases can lead to protein degradation, ii) heme can interfere with PCR, and iii) blood-derived proteins can be a confounding factor for quantifying effects in the CSF proteome.

Recommendations for Sample Preparation

Sample collection and preparation

- CSF should be collected using best practice clinical guidelines.
- Considerations for standard collection of CSF samples have been outlined by Teunissen et al. (*Neurology*, 2009, 73:1914–1922, DOI: 10.1212/WNL.0b013e3181c47cc2) and include:
 - Volume of withdrawal
 - Location of puncture
 - Use of polypropylene collection tubes
 - Time of day of withdrawal
- 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: It is not necessary to add protease inhibitors to samples.
- Samples should be centrifuged for 10 min at $\geq 500 \times g$ to remove cells and insoluble material.

- Aliquots should be stored at -80°C.

Note: Multiple samples from the same withdrawal should be pooled, mixed, and then aliquoted.

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

Campbell K, et al. Identification of cerebral spinal fluid protein biomarkers in Niemann-Pick disease, type C1. *Biomark Res.* 2023; 11(1):14. DOI: 10.1186/s40364-023-00448-x. [Link](#) [Olink Explore]

Wang H, et al. Evaluation of neurofilament light chain as a biomarker of neurodegeneration in X-linked childhood cerebral adrenoleukodystrophy. *Cells.* 2022; 11(5):913. DOI: 10.3390/cells11050913. [Link](#)

Göteson A, et al. Cerebrospinal fluid proteomics targeted for central nervous system processes in bipolar disorder. *Mol Psychiatry.* 2021; 26(12):7446-7453. DOI: 10.1038/s41380-021-01236-5. [Link](#)

Remsik J, et al. Inflammatory leptomeningeal cytokines mediate COVID-19 neurologic symptoms in cancer patients. *Cancer Cell.* 2021; 39(2):276-283.e3. DOI: 10.1016/j.ccell.2021.01.007. [Link](#)

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