Olink®

Running alternative matrices

Analysis of Tissue Samples

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 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.

In addition to plasma and serum, strategies have been developed to analyze alternative types of samples. For fresh tissue lysates, samples are normalized by protein concentration (0.5 mg/ml) and biological replicates should be included to account for technical differences in sample preparation. Technical replicates can also be added for better estimation of CVs when using an alternative matrix. To evaluate protein assays at risk for hook, it is recommended to run a few samples from each study group at two additional dilutions. Lysis buffer alone can be included to monitor background noise. Special attention should be paid to formulation of the lysis buffer, more information can be found in the document *Running alternative matrices: Buffer compatibility with Olink*.

Recommendations for Sample Preparation

General guidelines for lysing tissue samples

- Tissues are more difficult to lyse compared to *in vitro* cell culture, and require additional mechanical methods such as homogenizers, bead blenders, or sonicators.
- Follow manufacturer instructions for use of specific instrumentation and commercial lysis buffers.
- Keep your lysis buffer cold, perform lysis on ice, and centrifugations at 4°C.
- The general rule of thumb is that the ratio of tissue to lysis buffer should be in the range of 1:10 to 1:50 in g/ml. For example, 10 mg of tissue should be lysed in 100 µl to 500 µl of lysis buffer.
- Start with a higher ratio of tissue to lysis buffer to avoid diluting your protein concentration too low.
- Examine your samples during the mechanical lysis process to ensure that they are dissolving. It may be required to increase the strength otime of homogenization/blending/sonication, or to add more lysis buffer.
- After lysis is complete, samples should be centrifuged at high speed at 4°C to remove debris.
- The protein concentration of clarified lysates should be estimated using standard techniques (e.g., BCA, Bradford, Lowry, or Nanodrop assays).

- Samples should be normalized to a single protein concentration, such as 0.5 mg/ml, using the same lysis buffer. Use of other diluents, such as PBS or water, will cause the samples to have varying salt and detergent concentrations. Higher protein concentrations such as 1 mg/ml are also acceptable.
- Sample aliquots should be stored at -80°C or lower.
- To test the quality of your lysates, you can separate samples by SDS-PAGE and stain with Coomassie, silver, or fluorescent dyes. This will give information on protein integrity (i.e., clear banding vs smearing) as well as a check on the relative comparison of protein abundance.
- Please consult with support@link.com for formalin-fixed paraffin-embedded (FFPE) samples, or other tissue preparation protocols that include preservatives, fixatives, or denaturing agents.

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

Han J, et al. Modulation of inflammatory proteins in serum may reflect cutaneous immune responses in cancer immunotherapy. *JID Innov.* 2023; 3(2):100179. DOI: 10.1016/j.xjidi.2022.100179. Link [*skin tumor biopsies*]

Pavlović N, Kopsida M, Gerwins P, Heindryckx F. Activated platelets contribute to the progression of hepatocellular carcinoma by altering the tumor environment. *Life Sci.* 2021; 277:119612. DOI: 10.1016/j.lfs.2021.119612. Link [*mouse liver*]

Pavel AB, et al. The proteomic skin profile of moderate-to-severe atopic dermatitis patients shows an inflammatory signature. *J Am Acad Dermatol.* 2020; 82(3):690-699. DOI: 10.1016/j.jaad.2019.10.039. Link [*skin biopsies*]

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

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Olink Proteomics, Dag Hammarskjölds väg 52B , SE-752 37 Uppsala, Sweden
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