

# Analysis of Cell Culture Lysates

## Study Design Considerations

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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 cell culture 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 studying 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

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### General guidelines for lysing cell culture samples

- Follow manufacturer instructions for use of commercial lysis buffers or kits.
- Keep your lysis buffer cold, perform lysis on ice, and centrifugations at 4°C.
- Adherent cells or cells under suspension should be washed twice with cold PBS or HBSS to remove culture medium containing FBS or growth factors.
- The amount of lysis buffer that is added to the cell culture should be predetermined empirically, but in general it will be about 100 µl per 10<sup>6</sup> cells. The final protein concentrations of the cell lysates should all be >0.5 mg/ml so that they can be normalized to 0.5 mg/ml.

*Note:* Samples should be diluted using the same lysis buffer. Use of other diluents, such as PBS, will cause the samples to have varying salt and detergent concentrations

- For adherent cells, after removing excess wash solution by suction/pipet, lysis buffer can be added on top of the cells, swirled to ensure even dispersion, and then placed on ice for 10 min. Lysates can be scraped from the flask/dish surface using a cell scraper and transferred to a LoBind® Eppendorf microcentrifuge tube with a pipettor.

- For cells in suspension, washes can be performed with low speed centrifugation (500 x g for 5-10 min at 4°C). Excess wash solution can be removed from the pellet by carefully inverting the tube and then slowly aspirating from the liquid surface. After addition of lysis buffer to the cell pellet, the sample can be mixed by gentle vortexing or by pipetting up-and-down a few times. Samples can be incubated on ice for 5-10 min to complete cell lysis.
- 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).
- 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 relative comparison of protein abundance.

## Pre-Dilution Strategies

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### Target 96:

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

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Rosendal E, et al. Serine protease inhibitors restrict host susceptibility to SARS-CoV-2 infections. *mBio*. 2022; 13(3):e0089222. DOI: 10.1128/mbio.00892-22. [Link](#) [primary human bronchial epithelial cells]

Reimegård J, Tarbier M, Danielsson M, Schuster J, Baskaran S, Panagiotou S, Dahl N, Friedländer MR, Gallant CJ. A combined approach for single-cell mRNA and intracellular protein expression analysis. *Commun Biol*. 2021; 4(1):624. DOI: 10.1038/s42003-021-02142-w. [Link](#) [embryonic stem cell line]

Johansson P, et al. A patient-derived cell atlas informs precision targeting of glioblastoma. *Cell Rep*. 2020; 32(2):107897. DOI: 10.1016/j.celrep.2020.107897. [Link](#) [glioblastoma cell lines]

Please contact [support@olink.com](mailto:support@olink.com) for further information on running alternative matrices

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