

# Analysis of Lavage

## 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. 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. Lavage samples are collected by washing an internal body cavity with a buffered saline solution and then collecting the solution for analysis. Bronchoalveolar lavage (BAL) sampling is the most common technique, but other lavage samples we have analyzed include nasal and uterine.

Normalization of samples is typically performed by volume, therefore it is important to use a set volume when performing the lavage. Biological replicates are not necessary. Technical replicates can be included 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. The lavage buffer alone can be included as a control to monitor background levels.

## Recommendations for Sample Preparation

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- Ensure that salt concentrations of the lavage solution are within our guidelines ( $\leq 250$  mM NaCl,  $\leq 25$  mM KCl, and  $\leq 10$  mM  $\text{MgCl}_2$ )
- The pH of the lavage solution should be close to physiological levels (7-8 range)
- Following collection, keep samples on ice or at 4°C

*Optional:* You can add a protease inhibitor cocktail at this point, such as Roche cOmplete™ Mini Protease Inhibitor Cocktail (#11836153001)

- Centrifuge samples at high speed to remove cells and particulates
- Aliquot supernatant and store at -80°C

## 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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Vijayakumar B, et al. Immuno-proteomic profiling reveals aberrant immune cell regulation in the airways of individuals with ongoing post-COVID-19 respiratory disease. *Immunity*. 2022; 55(3):542-556.e5. DOI: 10.1016/j.immuni.2022.01.017. [Link](#) [BAL]

Verma A, et al. Monoclonal antibodies protect aged rhesus macaques from SARS-CoV-2-induced immune activation and neuroinflammation. *Cell Rep*. 2021; 37(5):109942. DOI: 10.1016/j.celrep.2021.109942. [Link](#) [BAL in NHP]

Bhargava M, et al. Bronchoalveolar lavage fluid protein expression in acute respiratory distress syndrome provides insights into pathways activated in subjects with different outcomes. *Sci Rep*. 2017; 7(1):7464. DOI: 10.1038/s41598-017-07791-8. [Link](#) [BAL]

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

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