

Running alternative matrices

# Analysis of Sputum

## 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. Sputum, also known as phlegm, is a thick type of mucus that is produced in the lungs. Sputum can be collected by either non-invasive or invasive methods. It can be used to investigate infectious and inflammatory airway diseases such as bronchitis, pneumonia, tuberculosis, COPD, and cystic fibrosis.

Normalization of samples can be based on either total protein concentration (e.g., 0.5 mg/ml) or volume. 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. It is necessary to keep samples cold and add protease inhibitors to minimize the effect of salivary proteases on protein integrity.

## Recommendations for Sample Preparation

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### Non-invasive collection protocol

#### *Materials and Equipment*

- Roche cOmplete™ Mini Protease Inhibitor Cocktail (#11836153001)
- PBS
- 50 ml conical tubes
- 1.5 ml LoBind® Eppendorf microcentrifuge tubes
- Refrigerated microcentrifuge
- Vortex
- Scale
- Ice bath or cold block

## Procedure

1. Advise the person to drink plenty of water the night before collection. This will help loosen the secretions and make it easier to cough up sputum.
2. Ask the person not to drink, eat, chew gum, and use nicotine or dental hygiene products 1 h prior to sample collection.
3. Collect samples at a standard time to avoid diurnal shifts in mucus production. The best quality of sample is usually obtained early in the morning.
4. The mouth should be free of foreign matter. If dentures are worn then they should be removed.
5. Ask the person to rinse their mouth with tap or sterile water and to spit out the water.
6. Ask the person to take several deep breaths through their mouth and then cough up mucous from deep within their lungs.

*Note:* Breathing deeply over steam may help raise sputum.

7. Ask the person to open a pre-weighed 50 ml conical tube and hold it close to their mouth, then transfer the mucous into the tube. Keep the sample on ice.

*Note:* 1-2 teaspoons of sputum is adequate.

8. Calculate sputum weight [= container/sputum weight – container weight], then add PBS equivalent to 8X sputum weight.
9. Add protease inhibitor cocktail to a 1X final concentration.
10. Incubate for 15 min while vortexing to break up the mucus.

*Optional:* Samples can be clarified through a 40 mm filter pre-soaked with PBS

11. Transfer 1 ml to a microcentrifuge tube and centrifuge at high speed for 5 min at 4°C.
12. Aliquot the supernatant and store at -80°C.

## 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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Blanco JR, et al. Elevated levels of serum CDCP1 in individuals recovering from severe COVID-19 disease. *Aging (Albany NY)*. 2022; 14(4):1597-1610. DOI: 10.18632/aging.203898. [Link](#)

Kasaian MT, Lee J, Brennan A, Danto SI, Black KE, Fitz L, Dixon AE. Proteomic analysis of serum and sputum analytes distinguishes controlled and poorly controlled asthmatics. *Clin Exp Allergy*. 2018; 48(7):814-824. DOI: 10.1111/cea.13151. [Link](#)

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

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