

Running alternative matrices

Analysis of Nasal Mucosa

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. 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. Here we describe two methods for obtaining nasal mucosal lining fluid: i) nasal strips based on a synthetic absorptive matrix (SAM™), and ii) nasal swabs. These minimally invasive sampling techniques can be used to investigate lung cancer as well as pulmonary, inflammatory, and infectious diseases such as asthma, allergic rhinitis, tuberculosis, and COPD. Samples are normalized by volume. It is not necessary to include biological replicates. Technical replicates can be included for better estimation of CVs when using an alternative matrix. A negative control containing elution buffer alone should be included to monitor background noise. To evaluate protein assays at risk for hook, it is recommended to run a few samples from each study group at two additional dilutions.

Recommendations for Sample Preparation

Nasal strips

Note: A detailed protocol description and video can be found here:

<https://www.jove.com/t/56413/absorption-nasal-bronchial-fluids-precision-sampling-human>

Materials and Equipment

- Nasosorption™ FX•i devices (Mucosal Diagnostics)
- Costar® Spin-X® Centrifuge Tube Filters (Product #8160)
- 15 ml conical tubes
- 0.5 ml or 1.5 ml Eppendorf LoBind® microcentrifuge tubes
- Sterile scalpel
- Sterile tweezers
- Protease inhibitor cocktail (Roche #11836153001)
- Microcentrifuge (speed up to 16,000 x g)

- Elution buffer; three options are:

1. 0.9% NaCl solution (AddiPak® #200-59)
2. PBS buffer with 0.05% Tween-20 and 1% BSA (Teknova #P0234)
3. 1% Triton X-100 or NP-40 with 1% BSA

Note: Triton buffer will lyse cells and enable both intracellular and extracellular proteins to be eluted, and generally result in higher levels of cytokines and chemokines.

Procedure

1. Prepare elution buffer with 1X protease inhibitors and store on ice.
2. Perform the nasal absorption sampling. Gently insert the SAM in the lumen of the nostril, orientating it flat against the inferior turbinate. Ask the person to use an index finger to press the SAM onto the nasal mucosa for 60 s.
3. Remove the SAM and return it to its original cryotube and proceed immediately to processing.

Note: Place devices on ice for short-term storage or at -80°C for long-term storage.

4. Detach the nasal SAM strip from the FX•i device at the point of perforation by using a sterile scalpel and transfer SAM into a Spin-X centrifuge tube using sterile tweezers.

Note: Clean scalpel and tweezers in between samples to avoid cross contamination.

5. Fold/twist the SAM strip into the tube so that it is pushed down as far down as possible.
6. Add 300 µl of cold elution buffer to the tube.

Note: Ensure that the SAM strip is completely covered by elution buffer.

7. Incubate samples at room temperature for 10 min.

Note: Samples can also be placed in a tube shaker.

8. Centrifuge tubes at 16,000 x g for 10 min at room temperature.
9. Aliquot eluates into 0.5 ml or 1.5 ml LoBind tubes and store at -80°C.

Nasal swabs

Materials and Equipment

- IVALON® Post-Op Sinus Packing (#Q770530)
- 15 ml conical tubes
- 0.5 ml or 1.5 ml LoBind® microcentrifuge tubes
- Sterile tweezers
- 5 cc plastic disposable syringes
- Elution buffer (see 3 options above)
- Protease inhibitor cocktail (Roche #11836153001)
- Refrigerated centrifuge for 15 ml conical tubes

Procedure

1. Prepare 10 ml of elution buffer with 1X protease inhibitors and store on ice.
2. Insert one IVALON nasal swab into each nostril in the region of the middle meatus for 5 min.
3. Remove swabs from nostrils and transfer to two 15 ml tubes with 3 ml of elution buffer. Push the swabs to the bottom of the tubes with tweezers so that they are completely immersed.
4. Cap the tubes and incubate for 2 h on ice or at 4°C.
5. Using tweezers, transfer the 2 swabs from the elution buffer into one 5 cc syringe barrel. Transfer both the remaining eluates and the 5 cc syringe with swabs into a new 15 ml conical.
6. Centrifuge the 15 ml tube with syringe barrel at 1500 x g for 15 min at 4°C (about 6 ml).
7. Discard the 5 cc syringe barrel with swabs into a medical waste container.
8. Aliquot eluates into 0.5 ml or 1.5 ml LoBind tubes and store at -80°C.

Pre-Dilution Strategies

Nasal Strips

Target 96:

CAM	CRE	CVDII	CVDIII	DEV	IMO	INF	IRE	MET	NEU	NEX	ODA	ONCII	ONCIII
1:64	1:4	1:4	1:16	1:16	1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4

Target 48:

1:4

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.

Nasal Swabs

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

Wimmers F, et al. Multi-omics analysis of mucosal and systemic immunity to SARS-CoV-2 after birth. *Cell*. 2023; 186(21):4632-4651.e23. DOI: 10.1016/j.cell.2023.08.044. [Link](#)

Martinson N, et al. Proteomic analysis of mucosal and systemic responses to SARS-CoV-2 antigen. *Vaccines (Basel)*. 2023; 11(2):334. DOI: 10.3390/vaccines11020334. [Link](#)

Zetlen HL, et al. Comparison of multiplexed protein analysis platforms for the detection of biomarkers in the nasal epithelial lining fluid of healthy subjects. *J Immunol Methods*. 2023; 517:113473. DOI: 10.1016/j.jim.2023.113473. [Link](#)

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