

Analysis of Saliva

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. Saliva is an attractive biological sample type since its inexpensive and non-invasive to collect. It can be used to investigate infectious, endocrine, and immunological diseases as well as cancer. It is important to be consistent in sample collection, processing, and storage. Collect the saliva sample from a standardized location within the mouth (either whole saliva or parotid saliva). Do not use an absorptive technique (e.g., swabs) for saliva sample collection unless its effect on the salivary proteome has been investigated.

The following should be assessed before collection of saliva samples:

- Recent history of alcohol, caffeine, and nicotine consumption
- Whether the individual has oral injuries, dental work, or oral disease
- Determine whether there is bleeding (visual inspection or transferrin assay)
- Survey the level of physical activity level prior to sample collection

Note: Excessive activity and dehydration can alter salivary flow rate and impact the proteomic profile of saliva

Normalization of samples can be based on either: i) volume (standard method); ii) total protein concentration (e.g., 0.5 mg/ml); or iii) salivary flow rate (ml/min) measured during sample collection (Ref 1). 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. Additional information on salivary proteomics and sample collection can be found in Ref 2.

[illegible]

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.

References

Ref 1: González-Hernández JM, et al. Influence of sampling conditions, salivary flow, and total protein content in uric acid measurements in saliva. *Antioxidants (Basel)*. 2019; 8(9):389. DOI: 10.3390/antiox8090389. [Link](#)

Ref 2: Bhattarai KR, Kim HR, Chae HJ. Compliance with saliva collection protocol in healthy volunteers: Strategies for managing risk and errors. *Int J Med Sci*. 2018; 15(8):823-831. DOI: 10.7150/ijms.25146. [Link](#)

Publications using Olink

Børsting T, Venkatraman V, Fagerhaug TN, Skeie MS, Stafne SN, Feuerherm AJ, Sen A. Systematic assessment of salivary inflammatory markers and dental caries in children: an exploratory study. *Acta Odontol Scand*. 2022; 80(5):338-345. DOI: 10.1080/00016357.2021.2011400. [Link](#)

Majster M, Lira-Junior R, Höög CM, Almer S, Boström EA. Salivary and serum inflammatory profiles reflect different aspects of inflammatory bowel disease activity. *Inflamm Bowel Dis*. 2020; 26(10):1588-1596. DOI: 10.1093/ibd/izaa190. [Link](#)

Di Pietro V, Porto E, Ragusa M, Barbagallo C, Davies D, Forcione M, Logan A, Di Pietro C, Purrello M, Grey M, Hammond D, Sawlani V, Barbey AK, Belli A. Salivary microRNAs: Diagnostic markers of mild traumatic brain injury in contact-sport. *Front Mol Neurosci*. 2018; 11:290. DOI: 10.3389/fnmol.2018.00290. [Link](#)

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