

Analysis of Urine

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 and it is best to use a balanced number of samples across the study groups. Replicates are not necessary.

In addition to plasma and serum, strategies have been developed to analyze alternative types of samples. Due to its variable contents, and high levels of salts and other metabolites, urine can potentially interfere with Olink's proximity extension assay (PEA) technology. Therefore, the standard procedure for running urine on Olink is to dilute samples at least 1:4 with Olink Diluent and normalize by volume. This method has been proven to have reproducible results that pass Olink's quality control.

An alternative approach would be to concentrate and wash urinary proteins using an Amicon® or Centricon® centrifugal filter: proteins can be eluted in PBS and normalized to a set concentration per sample (0.5 mg/ml is standard). However, true differences in protein abundance across different study groups may be normalized out. To evaluate protein assays at risk for hook, it is recommended to run a few samples from each study group at two additional dilutions. Technical replicates can also be included for better estimation of CVs when using an alternative matrix.

Recommendations for Sample Preparation

Sample collection

- Olink recommends using first morning urine for two reasons: i) it is usually more concentrated, and ii) this ensures that the urine samples are taken at a similar timepoint.
- An alternative approach would be to take aliquots from a combined 24 h collection.
- Fresh urine samples should be stored at 4°C until processing.
- Roche cOmplete™ Mini Protease Inhibitor Cocktail (#11836153001) is highly recommended.
- Aliquots should be stored at -80°C.
- Just prior to running Olink, samples should be thawed to room temperature, vortexed, and centrifuged for 10 min at $\geq 500 \times g$ to remove insoluble material. The supernatant is retained for the Incubation step.

Pre-Dilution Strategies

Target 96:

CAM	CRE	CVDII	CVDIII	DEV	IMO	INF	IRE	MET	NEU	NEX	ODA	ONCII	ONCIII
1:40	1:4	1:4	1:4	1:4	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.

Publications using Olink

Fellström B, Helmersson-Karlqvist J, Lind L, Soveri I, Thulin M, Ärnlov J, Kultima K, Larsson A. Albumin urinary excretion is associated with increased levels of urinary chemokines, cytokines, and growth factors levels in humans. *Biomolecules*. 2021; 11(3):396. DOI: 10.3390/biom11030396. [Link](#)

Ferreira JP, Rossignol P, Bakris G, Mehta C, White WB, Zannad F. Blood and urine biomarkers predicting worsening kidney function in patients with type 2 diabetes post-acute coronary syndrome: An analysis from the EXAMINE trial. *Am J Nephrol*. 2021; 52(12):969-976. DOI: 10.1159/000519436. [Link](#)

Gradin A, Andersson H, Luther T, Anderberg SB, Rubertsson S, Lipcsey M, Åberg M, Larsson A, Frithiof R, Hultström M. Urinary cytokines correlate with acute kidney injury in critically ill COVID-19 patients. *Cytokine*. 2021; 146:155589. DOI: 10.1016/j.cyto.2021.155589. [Link](#)

Please contact support@olink.com for further information on running standard matrices.

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Olink Proteomics, Dag Hammarskjölds väg 52B, SE-752 37 Uppsala, Sweden

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