

Running alternative matrices

Analysis of Tears

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. Tears are produced from the lacrimal gland located within the orbit of the eye. Tears are mostly composed of water but also contain salt, fatty oils, and over 1,500 unique proteins. Ocular fluids such as tears are useful for investigation of infectious and inflammatory eye conditions and diseases.

Tear samples can be collected by various methods such as Schirmer strips, microcapillary tube, absorbent-based methods such as sponges, and eye-flush. A Schirmer strip is a standard technique used in ophthalmology clinics and is a type of filter paper which is placed partially under the eyelid within the lower cul-de-sac. For capillary sampling, the tip of the glass capillary is inserted into the lower tear meniscus and tear fluid can be sampled from the conjunctival sac via capillary action. This method requires more experience by the clinician and is more invasive to the subject, but it collects less off-target proteins compared to Schirmer strips.

Note: Fluorescein is a commonly used diagnostic contrast agent for various ophthalmic procedures. If you plan on using Olink Target platforms, do not collect tears from patients who have been treated with fluorescein during the previous 24 h as it can interfere with qPCR.

Tears are normalized by volume. It is not necessary to include biological replicates or to add protease inhibitors. Technical replicates can be included for better estimation of CVs when using an alternative matrix. For Schirmer strips, 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

Tears – Schirmer strips

Materials and Equipment

- Schirmer strips (various manufacturers produce this product)
- Costar® Spin-X® Centrifuge Tube Filters (Product #8160)
- 15 ml conical tubes
- 0.5 ml or 1.5 ml Eppendorf LoBind® microcentrifuge tubes
- Sterile tweezers
- Sterile nitrile gloves
- Microcentrifuge
- Elution buffer; three options are:
 - 0.9% NaCl solution (AddiPak® #200-59)
 - PBS buffer with 0.05% Tween-20 and 1% BSA (Teknova #P0234)
 - 1% Triton X-100 or NP-40 with 1% BSA

Procedure

1. Perform the tear sampling with Schirmer strips according to the manufacturer's instructions.
2. Using sterile nitrile gloves, roll the Schirmer strip up and transfer to a Spin-X centrifuge tube with sterile tweezers. Push to the bottom. Proceed immediately to processing.

Note: Alternatively, you can use a section or a punch from the Schirmer strip. Be consistent from sample to sample.

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

Note: Alternatively, transfer Schirmer strips to 1.5 ml LoBind tubes and place on ice for short-term or -80°C for long-term storage.

3. Prepare stock of elution buffer in 15 ml conical.
4. Add 300 µl of elution buffer to the Spin-X centrifuge tube with Schirmer strip.

Note: Ensure that the Schirmer strip is completely covered by elution buffer.
5. Incubate samples at room temperature for 10 min.

Note: Alternatively, samples can be placed in a tube shaker.
6. Centrifuge tubes at 16,000 x g for 10 min at room temperature.
7. Aliquot eluates into 0.5 ml or 1.5 ml LoBind tubes and store at -80°C.

Pre-Dilution Strategies

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

Vergouwen DPC, et al. Evaluation of pre-processing methods for tear fluid proteomics using proximity extension assays. *Sci Rep.* 2023; 13(1):4433. DOI: 10.1038/s41598-023-31227-1. [Link](#)

Csősz É, Tóth N, Deák E, Csutak A, Tőzsér J. Wound-healing markers revealed by Proximity Extension Assay in tears of patients following glaucoma surgery. *Int J Mol Sci.* 2018; 19(12):4096. DOI: 10.3390/ijms19124096. [Link](#)

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

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