

# Analysis of Ocular Fluids

## 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. Here we describe methods for analyzing two types of ocular fluid: aqueous humor (AH) and vitreous humor (VH). AH is a clear fluid located at the front part of the eye that is responsible for providing nutrients and draining any excess material and waste from the eye. VH is a colorless, transparent, gel-like material located between the retina and the lens. It is mainly composed of water with addition of proteins, salts, sugar, and collagen. Ocular fluids are useful for investigation of infectious and inflammatory eye conditions and diseases.

AH has a watery consistency, but VH is a gel-like substance with high viscosity which can impair accurate pipetting. Two popular methods that are used to clarify VH samples are: i) high speed centrifugation (e.g., 12,000 rpm for 15 min), and ii) centrifugal filters (e.g., 0.22 µm GV Durapore® filter from Millipore).

Ocular fluids 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. 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

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### Sample collection and preparation

- Ocular fluids should be collected using best practice clinical guidelines.
- Freshly collected samples are stable for a short duration at room temperature but should be stored on ice or at 4°C if possible.
- Aliquots should be stored at -80°C.

## Pre-Dilution Strategies

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### Target 96:

CAM	CRE	CVDII	CVDIII	DEV	IMO	INF	IRE	MET	NEU	NEX	ODA	ONCII	ONCIII
1:100	1:1	1:1	1:10	1:10	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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Wilson S, et al. Correlation of aqueous, vitreous, and serum protein levels in patients with retinal diseases. *Transl Vis Sci Technol.* 2023; 12(11):9. DOI: 10.1167/tvst.12.11.9. [Link](#) [AH and VH]

Peng C-C, et al. Diagnostic aqueous humor proteome predicts metastatic potential in uveal melanoma. *Int J Mol Sci.* 2023; 24(7):6825. DOI: 10.3390/ijms24076825. [Link](#) [Aqueous humor]

Wierenga APA, et al. Aqueous humor biomarkers identify three prognostic groups in uveal melanoma. *Invest Ophthalmol Vis Sci.* 2019; 60(14):4740-7. DOI: 10.1167/iovs.19-28309. [Link](#) [Aqueous humor]

Schrijver B, et al. Vitreous proteomics, a gateway to improved understanding and stratification of diverse uveitis aetiologies. *Acta Ophthalmol.* 2022; 100(4):403-13. DOI: 10.1111/aos.14993. [Link](#) [Vitreous humor]

Lamy R, et al. Comparative analysis of multiplex platforms for detecting vitreous biomarkers in diabetic retinopathy. *Transl Vis Sci Technol.* 2020; 9(10):3. DOI: 10.1167/tvst.9.10.3. [Link](#) [Vitreous humor]

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

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