

# Analysis of Blister Fluid

## 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 study, all samples should be randomized across all plates and 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. Blister fluid, also known as wound exudate, is produced as a result of vasodilation during the early stages of healing. It is mainly composed of water, but also contains electrolytes, nutrients, waste products, and proteins such as inflammatory mediators, proteases such as matrix metalloproteinases (MMPs), and growth factors. Cells present include neutrophils, macrophages, and platelets.

Blister fluids should be clear to light yellow. Red or pink color is an indication of blood contamination which could be a confounding factor in proteomic analysis. Cloudiness or green color may be an indication of infection. Notes should be made on sample appearance and viscosity.

Samples are normalized by volume or by protein concentration (0.5 mg/ml). To evaluate protein assays at risk for hook, it is recommended to run a few samples from each study group at two additional dilutions. It is not necessary to include biological replicates. Technical replicates can be included for better estimation of CVs when using an alternative matrix. Addition of protease inhibitors is recommended due to the presence of proteases in blister fluids, particularly in cases of chronic wound healing.

## Recommendations for Sample Preparation

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### Sample collection

Wounds can result from accidents, medical procedures, insect and animal bites, infection, and disease conditions. In addition, they come in different sizes and shapes. Therefore, it is not possible to recommend a single sample preparation protocol. Please follow these general guidelines for use with Olink analysis:

- Samples should be collected as a liquid biofluid.
- There are various methods to aid in sample collection, such as syringes, vacuum/suction, drainage, or use of absorbent materials.
- Do not use an absorptive technique (e.g., swabs) for sample collection unless its effect on the blister fluid proteome has been investigated.

- Immediately after collection, samples should be placed on ice for short-term storage or at -80°C for long-term storage.
- Addition of a protease inhibitor cocktail is recommended, such as Roche cOmplete™ Mini Protease Inhibitor Cocktail (#11836153001). One tablet can be dissolved in 10 ml of lysis buffer. Alternatively, a 10X solution can be prepared by dissolving 1 tablet in 1 ml of distilled water or PBS, or a 7X stock in 1.5 ml. The stock solution can be stored at 4°C for ≤2 weeks or -20°C for ≤12 weeks. Use a 1X final concentration of inhibitor cocktail and avoid excess final concentrations (e.g., 2X or 3X).
- Samples should be collected in Eppendorf LoBind® microcentrifuge tubes and spun at high speed for 5 min to pellet cells and particulates.
- For dilutions, it is recommended to use Olink Diluent.

## Pre-Dilution Strategies

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### Target 96:

CAM	CRE	CVDII	CVDIII	DEV	IMO	INF	IRE	MET	NEU	NEX	ODA	ONCII	ONCIII
1:400	1:4	1:4	1:40	1:40	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

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Long Y, Li Y, Wang T, Ni A, Guo J, Dong Q, Yang S, Guo J, Wang L, Hou Z. Inflammation-related proteomics demonstrate landscape of fracture blister fluid in patients with acute compartment syndrome. *Front Immunol.* 2023; 14:1161479. DOI: 10.3389/fimmu.2023.1161479. [Link](#)

Clark KEN et al. Integrated analysis of dermal blister fluid proteomics and genome-wide skin gene expression in systemic sclerosis: an observational study. *Lancet Rheumatol.* 2022; 4:e507–16. DOI: 10.1016/S2665-9913(22)00094-7. [Link](#)

Sjöbom U, Christenson K, Hellström A, Nilsson AK. Inflammatory markers in suction blister fluid: A comparative study between interstitial fluid and plasma. *Front Immunol.* 2020; 11:597632. DOI: 10.3389/fimmu.2020.597632. [Link](#)

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

[www.olink.com](http://www.olink.com)

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Olink Proteomics, Dag Hammarskjölds väg 52B, SE-752 37 Uppsala, Sweden  
AM-16, v1.3