

Running alternative matrices

Analysis of Dried Blood Spots

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.

In addition to plasma and serum, strategies have been developed to analyze alternative types of samples. This includes dried blood spots (DBS) from whole blood. DBS are useful for collecting blood samples from infants for diagnostic purposes and are a convenient way to collect and store blood samples when it is not possible to maintain proper refrigeration. For long-term storage, DBS should be protected from moisture and stored at -20°C or lower, and eluates should be maintained at -80°C or lower. Replicates are recommended to account for technical differences during sample preparation (i.e., sample replicates using different punches). Technical replicates can also be added for better estimation of CVs when using an alternative matrix. Elution buffer alone should be included to monitor background noise. For DBS, normalization is based on a standard square area of filter paper used for sample collection, with the assumption that the area of filter paper equates to a standard volume of blood sample.

In addition to standard DBS samples, alternative collection procedures for whole blood sampling are described for Mitra® devices with VAMS® technology, Tasso-M20 devices, and Capitainer® qDBS.

Recommendations for Sample Preparation

Standard DBS Sample Prep

Paper Collection Cards

Olink has tested cards used for DBS collection. The cards that have proven to work well are:

- Whatman™ 903 filter paper
- Whatman™ FTA DMPK-C cards
- Ahlstrom-Munksjö BioSample TFN cards

The cards that showed suboptimal performance are Noviplex® plasma prep cards and PerkinElmer 226 Spot Saver cards. When testing other types of collection cards, our recommendations are to ensure that samples elute properly (i.e., minimal loss of diluent) and that samples dissolve well in the diluent (i.e., the eluate will turn dark pink/orange).

Materials and Equipment

- Samples collected on filter paper
- 2 ml LoBind® microcentrifuge tubes
- DBS card puncher, 3 mm
- 1X PBS
- TWEEN® 20
- Protease inhibitor cocktail (Roche #11836153001)
- Tube shaker

Optional:

- 96-well PCR plate with full skirt (e.g., Sarstedt #72.1980)
- 96-well plate seal (e.g., Life Technologies #4306311)

Procedure

1. Prepare 10 ml of elution buffer: 1X PBS with 0.05% TWEEN 20 and 1X protease inhibitors.
2. Using a 3 mm punch tool, punch the collection card where the blood has completely gone through the filter paper. Transfer the punch into a LoBind microcentrifuge tube.

Note: Take precautions not to contaminate the sample. For example, clean the punch tool and tweezers in between every sample to avoid cross contamination.

3. For every 3 mm punch, add 20 µl of elution buffer to the microcentrifuge tube.

Note: A 3 mm punch should contain approximately 3 µl of dried blood, roughly half of which will be plasma (the other half being cells).

Note: If preparing more than one 3 mm punch per sample, it is recommended to prepare in separate tubes and combine eluates after the elution step.

4. Ensure the punch is entirely covered by diluent and that it can easily move in the tube.
5. Close the lids and place the tubes on a shaker for 1 h at room temperature at about 600 rpm (or enough to see buffer movement).
6. Remove the samples from the shaker and transfer eluates to new tubes or to a 96-well plate.
7. Immediately freeze and store the samples at -80 °C.

Mitra devices with VAMS technology / Tasso-M20 Devices

Materials and Equipment

- Samples collected with 20 µL Mitra® Cartridges OR Tasso-M20 devices
- 2 ml round bottom LoBind® microcentrifuge tubes
- LoBind PCR strip tubes with individual caps
- 1X PBS
- TWEEN® 20
- Protease inhibitor cocktail (Roche #11836153001)
- Tube shaker

Procedure

1. Prepare 10 ml of elution buffer: 1X PBS with 0.05% TWEEN 20 and 1X protease inhibitor.
2. Carefully remove each individual VAMS tip from the Mitra cartridges OR each volumetric tip from the Tasso-M20 cartridges and place them in separate 2 ml LoBind microcentrifuge tubes.

Note: Take precautions against contaminating the samples. For example, clean the tweezers in between every sample.

Note: More detailed instructions for processing Tasso devices can be found here:

<https://www.tassoinc.com/tassom20-lab-instructions>.

3. For each 20 µl tip, add 180 µl of elution buffer to the microcentrifuge tube (this volume is sufficient to cover the tip entirely, and the tips should easily move in the tube).
4. Close the lid and place the tubes on a shaker for 1 h at room temperature at about 600 rpm (or enough to see buffer movement).
5. Remove the samples from the shaker and prepare 4 aliquots of 40-45 µl each in clearly labeled LoBind PCR strip tubes and tightly close each individual cap.
6. Immediately freeze and store the samples at -80°C.

Capitainer qDBS

This DBS microsampling technique enables at-home collection by using microfluidics to transfer 10 µl of blood to a collection membrane.

Materials and Equipment

- Samples collected on Capitainer® B cards
- Flat-bottom 96-well plates
- PBS with 0.05% TWEEN® 20
- Protease inhibitor cocktail (Roche #11836153001)
- Plate rocker
- Centrifuge that can hold 96-well plates

Procedure

1. Prepare 10 ml of elution buffer: 1X PBS with 0.05% TWEEN 20 and 1X protease inhibitor.
2. Eject discs containing DBS samples into separate wells of a flat-bottom 96-well plate.
3. Add 66 µl of elution buffer per well.
4. Rock the samples (170 rpm) for 1 h at room temperature.
5. Centrifuge plate at 3000 rpm for 3 min.
6. Transfer supernatant to a new 96-well plate and store at -80°C.

Pre-Dilution Strategies

Target 96:

CAM	CRE	CVDII	CVDIII	DEV	IMO	INF	IRE	MET	NEU	NEX	ODA	ONCII	ONCIII
1:100	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

van den Broek BTA, Lindemans CA, Boelens JJ, Delemarre EM, Drylewicz J, Verhoeven-Duif N, van Hasselt PM, Nierkens S. Long-term effect of hematopoietic cell transplantation on systemic inflammation in patients with mucopolysaccharidoses. *Blood Adv.* 2021; 5(16):3092-3101. DOI: 10.1182/bloodadvances.2020003824. [Link](#)

Björkesten J, et al. Stability of proteins in dried blood spot biobanks. *Mol Cell Proteomics.* 2017; 16(7):1286-1296. DOI: 10.1074/mcp.RA117.000015. [Link](#)

Fredolini C, et al. Precise blood proteome profiling in an undiagnosed population with COVID-19. *medRxiv.* 2021.11.15.21266315. DOI: 10.1101/2021.11.15.21266315. [Link](#) [Capitainer qDBS]

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

www.olink.com

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AM-02, v1.3