



Analysis of Mitra microsampling device with Olink

Olink has evaluated and optimized the protocol for Mitra microsampling device (<https://www.neoteryx.com/neoteryx-blood-collection-devices-microsampling-bio-fluids>)

SAMPLE COLLECTION

Collect blood sample from the subjects according to manufacturer's instructions

SAMPLE PREPARATION FOR OLINK ANALYSIS

Required Material and Equipment:

- 96-well PCR plate (compatible for up to 200 µL)
- 2 mL Round bottom LoBind microcentrifuge tubes (used for protein work): you want to make sure the VAMS tip has room to move in the tube
- LoBind PCR strip tubes with individual caps
- 1X PBS
- TWEEN20
- Protease inhibitor cocktail from Roche (cat number 11 836153001)
- Tube shaker
- 20µL Mitra Cartridges

Sample Preparation Methods:

1. Collect all your Mitra cartridge samples
2. Carefully remove each individual VAMS tip from the cartridge and place them in a separate LoBind microcentrifuge tube
3. Make sure not to introduce any contamination to the sample.
NOTE: If using a tweezer, make sure you clean the tweezers in between every sample to avoid cross contamination
4. Prepare 10 mL stock of elution buffer according to the following recipe:
 - 1X PBS + 0.05% TWEEN20 + protease inhibitor
 - NOTE: Prepare the solution and add the protease inhibitor freshly right before use. The working solution for protease inhibitor is 1 tablet/10 mL of the buffer hence suggesting to prepare a 10mL stock.

5. For every 20µL cartridge, add 180µl of elution buffer to the microcentrifuge tube (this volume is enough to cover the tip entirely)
6. Make sure the volume covers the entire VAMS tip and the tip can easily move in the tube
7. Close the lid and place the tubes on a shaker for 1 h at RT at 528 rpm (or 600 rpm; enough to see the buffer movement)
8. Once done, remove the samples from the shaker and prepare four aliquotes of 40-45 µL in clearly labeled Lobind PCR strip tubes and close each individual cap.
NOTE: Make sure that each individual tube is completely closed
9. Immediately transfer the aliquots into -80°C freezer until ready to ship the samples to Olink

OLINK INSTRUCTION

1. Upon receipt of samples, store samples immediately in -80°C freezer
2. When ready to run follow the dilutions below for different panels:

Panel	Olink Dilution **	Final Target Dilution
CAM	Dilute 110x (i.e. 1 µl of sample + 109 µl of sample diluent)	1:2025
CVD3, DEV	Dilute 5.5x (i.e. 1 µl of sample + 4.5 µl of sample diluent)	1:100
All others	Run without any dilution	

** Please note the above dilutions protocol are optimized for 20µL Mitra Cartridges. For other types of Mitra cartridges (10 µL or 30µL), use the below calculations to update dilution factors.

Dilution factor calculation: A 20 µl VAMS tip should, contain approximately 20 µl dried blood, roughly half of which will be plasma (the other half being cells) → 10 µl plasma → Olink elution step dilutes the sample 18x in terms of the plasma content so adjust dilution factors for CVD3, DEV and CAM accordingly.