Sample Preparation Instructions

Whole blood (human/animal)

Human

- Blood should be collected into the smallest EDTA (or heparin) vacutainer (2 mL)
- IMPORTANT! If possible, the blood samples should be divided into **150 - 500 µL** aliquots in separate test tubes, e.g. Eppendorf tube

Animal

- Blood should be collected into the EDTA (or heparin) tubes and divided into **120 - 200 µL** aliquots in separate test tubes, e.g. Eppendorf tube

All blood samples should be frozen at -20 °C within 4 - 6h after withdrawal for several days. Samples should be stored for longer time in -80 °C.
IMPORTANT! Keep consistent time between withdrawal and freezing for all samples.

PLEASE NOTE:

- **120 µL** of whole blood (human or animal) is enough for the measurement of 2 NAD metabolites using our kits or all 6 metabolites (NAD+, NADH, NADP+, NADPH, GSSG and GSH) using our laboratory service
- Plasma or serum are not suitable for NADMED technology
- NAD levels are normalized per volume (final concentration is in µM)
- Optional: NAD levels can be normalized per protein amount

Tissues (human/animal)

IMPORTANT: to reduce variability between the samples from different subjects/animals, it is very important to take aliquots of tissue from exactly same areas of the organ.

Fresh tissue samples

- Organ/tissue samples should be collected by standard method, rinsed with cold PBS and the excess of buffer removed with a paper towel
- Each organ/tissue sample should be approximately **15 - 25 mg**, the exact weight of each sample piece should be recorded
- Samples should be snap frozen in liquid nitrogen and stored in -80°C.

Frozen tissue samples

- If samples need to be aliquoted, it should be done in a frozen state to avoid sample melting
- The weight of each frozen sample should be recorded
- Samples should be stored in -80°C

PLEASE NOTE:

- NAD levels are normalized per sample weight
- Optional: NAD levels can be normalized per protein amount

Cultured cells

- One 10 cm plate (confluency 85 - 90%) or **~ 1.5M cells*** is enough for the measurement of 2 NAD metabolites using our kits or all 6 metabolites (NAD+, NADH, NADP+, NADPH, GSSG and GSH) using our laboratory service
- Cells should be grown in 10 cm plates until 85 - 90% confluency, then washed with excess of PBS
- Cells should be collected by scraping in PBS (**not trypsin**) and centrifuged (750 rpm)
- After removing the supernatant, cells should be snap frozen in liquid nitrogen and stored at -80°C
- NAD levels are normalized per protein amount

LABORATORY SERVICE

Please fill in the Service incoming form when preparing samples for the analysis at NADMED laboratory. For animal samples coming from outside EU, we need to apply for animal import permit before the samples can be shipped to Finland.

Pseudonymisation

All samples should be pseudonymized and labelled only with **sample-specific code**. We also recommend to randomize the order of the samples. Please provide us with basic information for each sample in a separate excel sheet that includes:

- Sample code
- Sample type (e.g. muscle)
- Sample volume or weight

Shipment

Samples should be shipped on **dry ice**. The amount should be sufficient enough to keep the sample frozen for several days. We recommend **2 kg/day** (EU) or **3 kg/day** (USA, Canada, Australia and Asia).

Shipping address:

NADMED Ltd. / laboratory
Biomedicum 1
Haartmaninkatu 8
00290 Helsinki
FINLAND

+358 41 312 4256
laboratory@nadmed.fi

* Depends on the cell type.