Sample Preparation Instructions

Whole blood (human/animal)

Human

- Blood should be collected into the EDTA (or heparin) vacutainer (purple cap) or with TASSO+ device© and **properly mixed.**
- IMPORTANT! Samples can be analyzed fresh or frozen. Fresh blood can be analyzed within 72 hours if maintained at 4 – 8°C.
- Fresh samples can be divided into **120 - 200 µL** aliquots in separate **single-wall microtubes** within 72 hours and then stored frozen at -20°C for one month or at -80°C for approximately a year.

Animal

- Blood should be collected into the EDTA (or heparin) tubes and **properly mixed.**
- Within 4 – 6 hours after withdrawal, blood samples should be divided into. **120 - 200 µL** aliquots in separate **single-wall microtubes,** and then stored frozen at -20°C for one month or for extended time periods at -80°C.
- IMPORTANT! For comparison of samples within a cohort, it is important to maintain similar time from sampling to 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, because most NADs exist inside the blood cells.
- NAD levels are normalized per volume (final concentration is in µM).
- Optional: NAD levels can be normalized per protein amount (additional costs per sample).

Tissues (human/animal)

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

Fresh/frozen 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.
- If samples need to be aliquoted, it should be done in a frozen state to avoid sample melting, and the weight of each frozen sample aliquot should be recorded.
- If the weight of each sample is not provided, additional costs will be charged for obtaining the weight at NADMED´s laboratory.

PLEASE NOTE:

- **15 - 25 mg** of a tissue is enough for the measurement of all 6 metabolites (NAD+, NADH, NADP+, NADPH, GSSG and GSH) using our laboratory service.
- NAD levels are normalized per sample weight
- Optional: NAD levels can be normalized per protein amount (additional costs per sample)

Cultured cells

- One 10 cm plate (confluency 85 - 90%) or **~ 1.5 million cells*** is enough for the measurement 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
Sonja Jansson
Biomedicum 1
Haartmaninkatu 8
00290 Helsinki
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Email: laboratory@nadmed.com

* Depends on the cell type