

Instructions for preparation of blood samples for the NADMED test

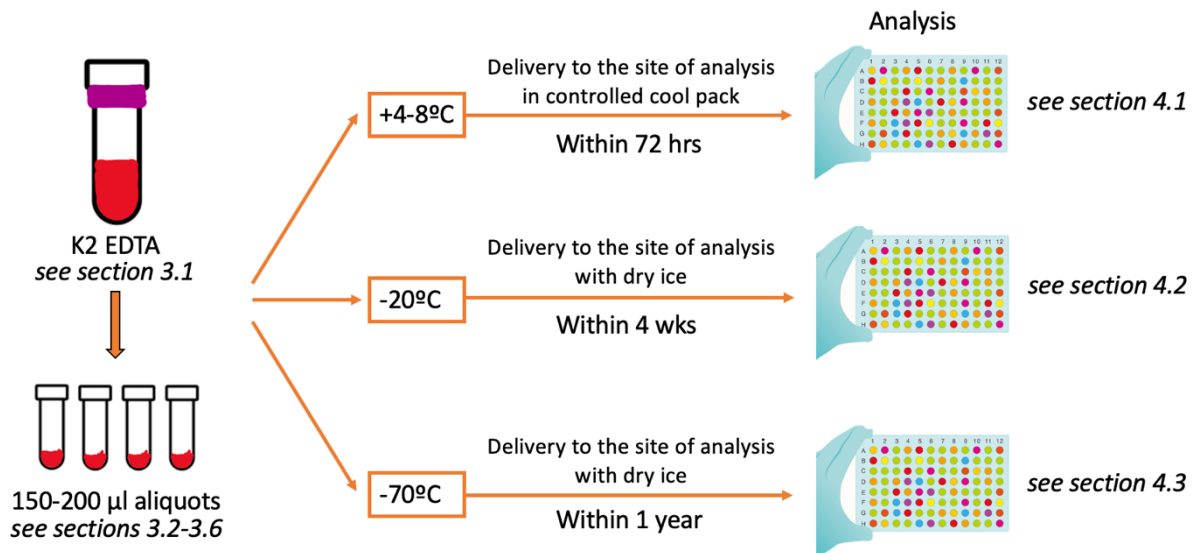
Version 2.0

1. General information

NADMED is a proprietary technology for analyzing REDOX metabolites. With our kits, you can individually measure NAD⁺ and NADH (or only NAD⁺) from a single sample. In our laboratory, we can perform individual measurements of NAD⁺, NADH, NADP⁺, NADPH, and reduced and oxidized glutathiones from a single sample. The customer defines whether all six metabolites or only selected ones will be measured. The result values are normalized per volume of whole blood and represented in μM units.

2. Information on the blood collection procedure

- There is no need for fasting before blood collection for the NADMED analysis.
- Whole blood can be collected by vein puncture by an authorized phlebotomist.
- Peripheral blood or capillary blood can be self-collected using lancet-type blood collection devices.
- Whole blood samples should be collected into collection tubes spray-coated with K2 EDTA or lithium heparin as anticoagulants and properly mixed by up-and-down rotation (180°).



3. Instructions for blood collection

- 3.1 Use K2 EDTA blood tubes containing spray-coated K2 EDTA resulting in a concentration of 1.2–2 mg of K2 EDTA per 1 mL of collected blood, or Lithium Heparin (LH) blood tubes containing spray-coated LH resulting in a concentration of 17–18 IU of LH per 1 mL of collected blood. The NADMED analysis requires small volumes of whole blood; thus, we recommend using small blood collection tubes, e.g., 2–3 mL. Collecting the intended volume of blood into the collection tube is vital to keep the target concentration of anticoagulant in the sample. Mix the collected sample by a few up-and-down rotation cycles (180°).
If a small blood volume (e.g., 200–500 µL) is collected with the self-sampling device using the K2 EDTA or LH tube, no aliquoting is needed.
- 3.2 The NADMED assay needs one aliquot of 150–200 µL of whole blood for measuring. Make aliquots of the collected samples for analysis and freeze them for storage and shipment if necessary. Decide how many aliquots you would like to prepare for every sample. Samples should be equilibrated to ambient temperature and carefully mixed before aliquoting to ensure an equal cell amount in each aliquot.
- 3.3 If a sample is aliquoted within one hour after collection, it can be kept at room temperature until the aliquoting procedure. Carefully mix the sample tube by up-and-down rotation (180°) before aliquoting to ensure the homogeneity of the sample.
- 3.4 If more time is needed before making the aliquots, keep the sample refrigerated at 4–8°C until aliquoting, but not longer than 72 hours after the draw. For clinical trials, we strongly recommend keeping the same time interval between sample draw and aliquoting/freezing for all the samples in the study.
- 3.5 To make the blood aliquots, use basic non-sterile single-wall 1.5–2 mL microcentrifuge tubes or tubes intended for blood storage. Do not use double-wall or skirted tubes as they slow down the process of sample thawing for analysis, possibly causing variability in results.
- 3.6 Before aliquoting the refrigerated samples, equilibrate the blood to room temperature. Mix carefully by several rounds of up-and-down rotation (180°) until the sample is homogeneous and immediately proceed with aliquoting of 150–200 µL of whole blood per tube.

4. Instructions for sample storage/shipping

- 4.1 Storage at 4–8°C: The samples can be stored and sent/delivered for up to 72 hours after the draw in 4–8°C. Sending/delivery of the samples needs to be done with controlled cool packs. Note that the samples need to be analyzed or frozen for longer storage after 72 hours to keep the metabolites intact.

4.2 Storage at -20°C: The samples can be frozen for short-term storage at -20°C for max a month. Sending/delivery of the samples needs to be done with dry ice to keep the metabolites intact. The amount of dry ice should be sufficient for the entire shipment time.

4.3 Storage at -70°C: The samples can be stored at -70°C for longer periods of time exceeding 1 month. There is no need to freeze samples in liquid nitrogen, but if you decide to do so, use single-wall tubes with screw caps (for example, NUNC). Sending/delivery of the samples needs to be done with dry ice to keep the metabolites intact. The amount of dry ice should be sufficient for the entire shipment time.

5. Handling retrospective samples that were stored frozen in large volumes

Samples frozen in large volumes are suitable for measurement only if they were always stored frozen. They can be sent to the NADMED facility with dry ice. Thawing the samples for making an aliquot for the NADMED test is **not allowed**. Please contact NADMED laboratory for more details or special requirements to return the sample leftovers.

PLEASE NOTE:

- **150 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 sample type for NAD measurement. All blood NADs reside in 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).

6. Laboratory service

Please fill in the Service incoming form when preparing samples for the analysis at the NADMED laboratory. We need to apply for an animal import permit for animal samples coming outside of the EU before the samples can be shipped to Finland. Please check with the NADMED laboratory that your country is on our import permit list **before** sending the samples.

7. Pseudonymization

All samples should be pseudonymized and labeled only with a **sample-specific code**. We also recommend randomizing 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. whole blood)
- Sample volume



8. Shipment to NADMED lab

Samples should be shipped with **dry ice**. The amount must be sufficient to keep the sample frozen for several days. We recommend **3 kg/day**. **For non-EU countries, please add a few extra days for possible customs delays.**

Shipping address:

NADMED Ltd. / laboratory

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