NADMED

Version 4.0

Instructions for Blood Sample Preparation for the NADMED analysis

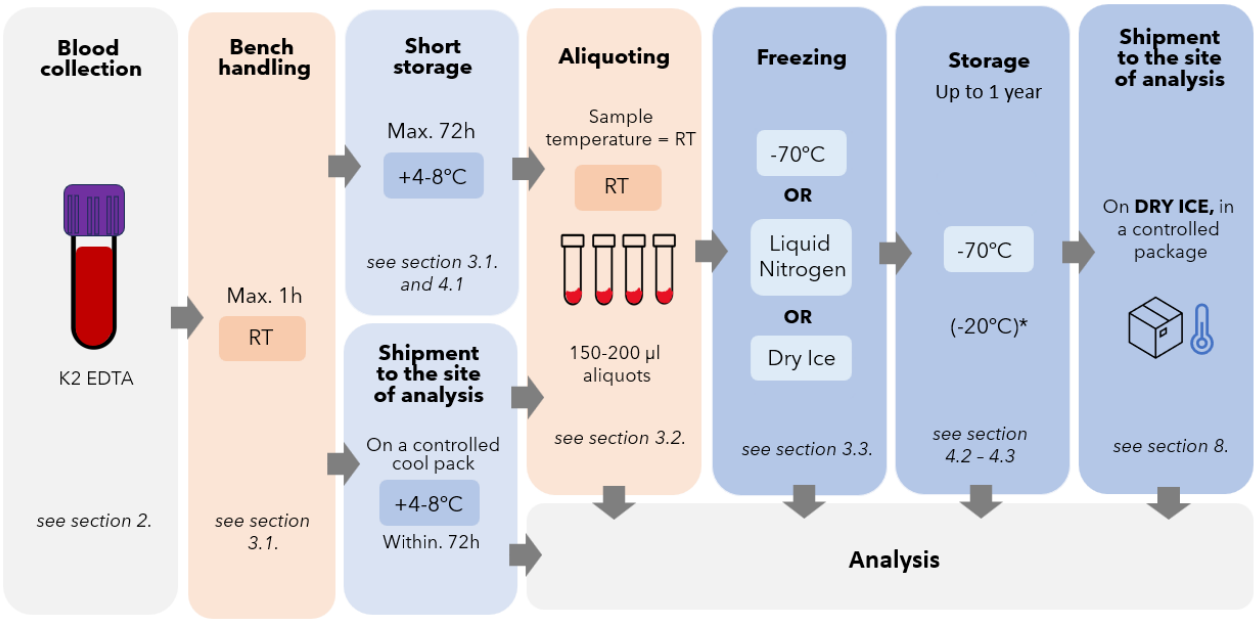
1. General information

NADMED has developed a proprietary technology for analyzing REDOX metabolites. A clinical laboratory can analyze both NAD+ and NADH from whole blood samples using our Q-NADMED Blood NAD+ and NADH Assay kits. In our laboratory, we can perform individual measurements of NAD+, NADH, NADP+, NADPH, and reduced (GSH) and oxidized (GSSG) glutathiones from a single sample. The customer specifies the number of metabolites to be analyzed per sample. Each metabolite measurement is normalized to whole-blood volume (µM) and compared with NADMED's established reference range.

This metabolite-focused sample handling framework is specifically designed to preserve the stability and integrity of the targeted analytes.

Preparation for the blood collection procedure

- To minimize biological variability, the sampling conditions should be standardized across individuals to be tested. We recommend applying a consistent protocol for all individuals, including fasting status (no fasting needed but can be applied if other needs), time of day of sample collection, and relevant pre-sampling factors such as recent physical activity. Ensuring comparable sampling conditions across subjects supports data consistency and interpretability.
- Whole blood can be collected by an authorized phlebotomist via venipuncture.
- Venous blood is preferred to ensure optimal sample integrity. However, peripheral or capillary blood may also be used, including self-collected samples obtained with lancet-based devices, provided that standardized collection procedures (e.g., appropriate timing, handling, and processing conditions) are strictly followed.



*Schematic picture of the options on the pre-analytical process flow. * -20°C should be used only for short-term storage, max. 1 month. See section 3.3. (Room temperature = RT)*

2. Blood collection

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. Drawing the specified volume of blood into the collection tube is essential to ensure the correct anticoagulant-to-blood ratio and maintain sample integrity. Mix the collected sample immediately with a few up-and-down rotation cycles (180°). Only a small volume of blood is required for NADMED analysis; the collected sample should be aliquoted into appropriately labeled, low-volume secondary tubes in accordance with the defined time frames, temperature conditions, and handling instructions described in Section 3.

3. Sample Aliquoting and Freezing

The NADMED assay needs one aliquot of 150–200 µL of whole blood. We recommend preparing several aliquots for each sample.

3.1. Time from blood collection to aliquoting

We strongly recommend maintaining the same time interval between sample draw, aliquoting, and freezing for all samples in the study.

Immediate post-collection aliquoting: If a sample is aliquoted within one hour after collection, it can be kept at room temperature until the aliquoting procedure.
Delayed Aliquoting with Refrigeration: If aliquoting cannot be performed within 1 hour of blood collection, keep the sample refrigerated at 4–8°C until aliquoting, but no longer than 72 hours after the draw.

3.2. Aliquoting procedure

Immediate post-collection aliquoting: Mix the sample carefully by several rounds of up-and-down rotation (180°) until homogeneous, then immediately aliquot 150–200 µL of whole blood per tube. 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. It is recommended to prepare aliquots in sets of three. Gently mix the primary sample before proceeding to the next set of aliquots to ensure homogeneity.

Aliquoting refrigerated samples: If samples were stored in a refrigerator before aliquoting, equilibrate the blood to room temperature (20 min at room temperature). Mix carefully by several rounds of up-and-down rotations (180°) until the sample is homogeneous, then immediately aliquot 150–200 µL of whole blood per tube.

3.3. Freezing of the samples

Freeze the samples immediately in a deep freezer (-80°C), in liquid nitrogen, or by placing them on dry ice.

Note: If you decide to freeze the samples in liquid nitrogen, use single-wall tubes with screw caps (for example, NUNC).

Note: Freezing at -20°C is not recommended, as the process is slower and potentially affects the sample integrity. However, if there is no other possibility, special attention is needed: -20°C should be used only for short-term storage (max. 1 month). Use pre-frozen racks to which you place the readily prepared aliquots (150-200 µL), and make sure to use a freezer with low traffic to minimize temperature fluctuations as much as possible.

4. Instructions for sample storage

4.1. Storage at 4–8°C up to 72 hours

Samples may be stored and transported at 4–8 °C for up to 72 hours following blood draw. Shipment must be carried out using temperature-controlled cool packs to maintain this range. For storage exceeding 72 hours, samples must be frozen to preserve metabolite stability. If aliquoting is required, it must be completed before freezing.

4.2. Storage at -70°C

Samples are stable during long-term storage at −70 °C for at least up to one year.

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. Thawing the samples for aliquoting for the NADMED test is not allowed.

PLEASE NOTE:

- A minimum of 150 µL of whole blood (human or animal) is required to ensure sufficient sample volume for comprehensive metabolite profiling. Although approximately 100 µL is used for the extraction process itself, additional volume is necessary to account for handling losses (e.g., during pipetting and mixing) and to ensure accurate and reproducible quantification. Using 150 µL ensures sufficient material for reliable measurement of two metabolites (NAD^+ and NADH) with our kits and of six metabolites (NAD^+, NADH, $NADP^+$, NADPH, GSSG, and GSH) through our laboratory service.
- Plasma or serum is not a suitable sample type for NAD measurement. The vast majority of NAD^+ and NADH in blood is intracellular and primarily

localized within red blood cells. Therefore, whole blood is required for accurate assessment of total blood NAD levels.
- NAD levels are normalized per volume (final concentration is in µM).

6. Laboratory service

First-time customers, please contact us at info@nadmed.com, and we'll help you to get started. Existing customers can sign in at www.shop.nadmed.com and create an order when preparing samples for the analysis at the NADMED laboratory.

7. Pseudonymization if using NADMED laboratory services

All samples should be pseudonymized and labeled only with a **sample-specific code**. We also recommend randomising the sample order. Please provide the sample details in the *Extra Info* step in the webshop. In addition, kindly print the corresponding sales order or quotation and include it with the shipment. This should include:

- Ordered service product
- Sample names

8. Shipment to NADMED lab

Samples should be shipped with **dry ice** to maintain metabolite integrity. The amount of dry ice must be sufficient to keep the sample frozen throughout the entire shipment. We recommend **3 kg/24h at ambient temperature. For non-EU countries, please add a few extra days for possible customs delays.** We recommend shipping on Mondays.

Shipping address:
NADMED Ltd. / Attn: Sonja Jansson
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