

Instructions for preparation of tissue and cell 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 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. Fresh/frozen 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.

- Organ/tissue samples should be collected by a standard method, rinsed with cold PBS, and the excess buffer removed with a paper towel.
- Each organ/tissue sample should be approximately 10 - 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:

- 10–20 mg of tissue is enough for the measurement of 2 metabolites (NAD⁺ and NADH) using our kit (RUO_003).
- 20–25 mg of 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).

3. Cultured cells

- One 10 cm plate (confluency 85 - 90%) or ~ 1.5–2.0 million cells* is enough for enough for the measurement of 2 metabolites (NAD⁺ and NADH) using our kit (RUO_003).

- ~ 2–3 million cells* is enough for the measurement of 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.

4. 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.

5. 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 weight OR number of cells

6. 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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Haartmaninkatu 8
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* Depends on the cell type