

# Instructions for Tissue and Cells Sample Preparation for the NADMED analysis

## 1. General information

NADMED has developed a proprietary technology for analyzing REDOX metabolites from biological samples. Both NAD<sup>+</sup> and NADH can be analyzed from a single sample using our RUO NAD<sup>+</sup> and NADH Assay kits. In our laboratory, we can perform individual measurements of NAD<sup>+</sup>, NADH, NADP<sup>+</sup>, 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 per protein amount.

This metabolite-focused sample handling framework is specifically designed to preserve the stability and integrity of the target analytes, supporting data consistency and interpretability. To minimize biological variability, the sampling conditions should be standardized across cells or tissues to be tested.

## 2. Fresh/frozen tissues (human/animal)

**IMPORTANT:** To reduce variability between samples from different subjects/animals, it is essential to take tissue aliquots from the exact same area of the organ and, preferably, include control samples.

- Organ/tissue samples should be collected by a standard method, rinsed with cold PBS, and the excess buffer liquid 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 and marked on the sample list.
- Samples should be snap-frozen in liquid nitrogen and stored in -70°C.
- If samples need to be aliquoted after freezing, it must 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<sup>+</sup> and NADH) using our assay kit (RUO\_003).
- 10-25 mg of tissue\* (muscle, liver, heart) is enough for the measurement of all 6 metabolites (NAD<sup>+</sup>, NADH, NADP<sup>+</sup>, NADPH, GSSG, and GSH) using our laboratory service. For other tissues please consult NADMED laboratory since the weight needed for analysis of 6 metabolites is tissue-specific.
- Quantified levels of the metabolites are normalized per protein amount.

*\*Dependes on the tissue type. 10-25 mg is sufficient for 6 metabolites in muscle, liver and heart tissue. For other tissues, please consult NADMED laboratory.*

### 3. Cultured cells

One 10 cm plate (confluency 85 - 90%) or ~ 1.5-2.0 million cells\* is enough for the measurement of 2 metabolites (NAD<sup>+</sup> and NADH) using our kit (RUO\_003).

- ~ 2-3 million cells\* is enough for the measurement of all 6 metabolites (NAD<sup>+</sup>, NADH, NADP<sup>+</sup>, NADPH, GSSG, and GSH) using our laboratory service.
- Cells should be grown in 10 cm plates until 85 - 90% confluency, then washed with a prewarmed (+37°C) excess of PBS to remove proteins.
- Cells should be collected by scraping in a prewarmed (50-55°C) extraction buffer (buffer B, included in the RUO\_003 kit) and centrifuged (750 rpm, 5min). If you are planning to send the cell sample to the NADMED laboratory, consult NADMED while planning, and you'll be provided with an extraction buffer (do not use a cell-detaching enzyme!).
- After removing the supernatant, cells should be snap-frozen in liquid nitrogen and stored at -70°C.
- Quantified levels of the metabolites are normalized per protein amount.

*\*Depends on the cell type: 2-3 million cells are sufficient in cultured fibroblasts and HeLa cells as reference. For other cell types, consult NADMED laboratory.*

### 4. Instructions for sample storage

#### 4.1. Storage at -70°C

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

## 5. Laboratory service

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

In case of animal samples, we need to apply for an animal sample import permit. Please refer to section 7.1.

## 6. Pseudonymization if using NADMED laboratory services

All samples must 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
- Sample type (e.g., tissue type).

## 7. 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.

### 7.1. Shipping animal samples

Animal samples sent to the NADMED laboratory **from outside the EU** require an **import permit**. Before shipping, please contact our laboratory at [laboratory@nadmed.com](mailto:laboratory@nadmed.com) and provide:

- Sample country of origin
- Animal species
- Sample type.

**Do not ship animal samples before receiving confirmation from us.** If our existing permit does not cover your samples, we will need to apply for a new one, which may cause a delay of up to **2 months**. The import permit must be printed and attached to the document pouch on the shipment.

**Shipping address:**

NADMED Ltd. / Attn: Sonja Jansson  
Biomedicum 2U, Tukholmankatu 8  
00290 Helsinki  
FINLAND  
Phone: +358 10 375 1181  
Email: [laboratory@nadmed.com](mailto:laboratory@nadmed.com)