NADMED

Sample Preparation Instructions

Whole blood (human)
-Blood should be collected into the smallest EDTA vacutainer (2 ml)
-IMPORTANT! If possible, the blood sample could be divided into 500 µl sample aliquots in separate tubes, e.g. Eppendorf tube
-Samples should be frozen at -20 °C as soon as possible after withdrawal for several days. Samples should be stored for longer time in -80 °C
IMPORTANT! Keep consistent time between withdrawal and freezing for all samples

Whole blood (animal)
-Blood should be collected into the EDTA tubes and divided into 200 µl sample aliquots in separate tubes, e.g. Eppendorf tube
-Samples should be frozen at -20°C as soon as possible after withdrawal for several days.
-Samples should be stored for longer time in -80°C

IMPORTANT! Keep consistent time between withdrawal and freezing for all samples

PLEASE NOTE:
-2 ml/500 µl (human) or 200 µl (animal) of whole blood is enough for the measurement of all four NAD metabolites
-Plasma or serum are not suitable for NADmed analysis
-NAD levels are normalized per volume (final concentration is in µM) .
-Optional: NAD levels can be normalized per protein amount .

Tissues (human/animal)
IMPORTANT: to reduce variability between the samples from different subjects/animals, it is very important to take aliquots of tissue from exactly same areas of the organ .

Fresh tissue samples:
-Organ/tissue sample should be collected by standard method, rinsed with cold PBS and the excess of buffer removed with paper towel
-Each organ/tissue sample should be 10-20 mg, the exact weight of each sample piece should be recorded Samples should be snap frozen in liquid nitrogen and stored in -80°C.

Frozen tissue samples:
If samples need to be aliquoted, it should be done under liquid nitrogen to avoid sample melting
The weight of each frozen sample should be recorded Samples should be stored in -80 °C
PLEASE NOTE: NAD levels are normalized per sample weight
Optional: NAD levels can be normalized per protein amount

Cultured cells
-One 10 cm plate (confluency 90-100 %) is suitable for the measurement of all four NAD metabolites
-Cells should be grown in 10 cm plates until 90-100 % 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, snap frozen in liquid nitrogen and stored at -80 °C
-NAD levels are normalized per protein amount

Pseudonymisation
All samples should be pseudonymized and labelled only with **sample-specific code**. We also recommend to randomize the order of the samples. Basic information for each sample required in a separate sheet (Sample code, Sample source (e.g. human) , Sample type (e.g. whole blood) and volume or weight of each sample) .

Shipment Samples should be shipped on **dry ice**. The amount should be sufficient enough to keep the sample frozen for several days. The institute is open on week days 7-19.

Shipping address: Liliya Euro/Wartiovaara lab Biomedicum 1 Haartmaninkatu 8 00290 Helsinki Finland

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