

DNA EXTRACTION

Responsible(s):

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SOP.BIO.004 – DNA EXTRACTION

1. SCOPE

This Standard Operating Procedure defines the isolation and storage protocols of DNA extracted from blood, which will be preserved at the Biobanco-IMM.

The DNA isolation will be performed only from samples from donors who have freely given their informed consent to the sample collection and storage at the Biobanco-IMM.

2. SAFETY INFORMATION

All specimens should be treated as infectious and handled according to “standard precautions”. Specimens must be processed only by trained staff. White coat, gloves, safety glasses and other individual protection devices must always be worn while collecting and handling samples.

A. Interferences

Although is strongly recommended to collect blood in vacuum K2EDTA for DNA extraction, blood collected in citrate or heparin can also be used.

4. DESCRIPTION / PROCEDURE

A. Specimen identification

The patient’s specimens must be unambiguously identified at the time of collection. Specimens should be labeled and handled in a manner that respects patient privacy according the law n.º 12/2005, published at *Diário da República*.

Each tube must be labeled with an identifier that links it with the donor’s unique identification number ensuring traceability of the specimen and separation of personal and clinic data.

B. Blood collection

For the material and method in peripheral blood collection see SOP02.

The informed consent and a questionnaire containing: identification and diagnosis (when applicable) of the donor should be sent together with the samples. In addition the following information should be also recorded: date and time of collection and shipment; name and signature of the person which collected the sample; amount and type of tubes; approximate temperature of transport and notes.

C. Processing procedure

i) Check that all specimens and relative documentation are available. If something is missing or if the documentation or accompanying labels are incomplete, illegible or mismatched contact the collection center. In this case, the Biobanco-IMM will put the samples on “stand-by”. If the missing information is not sent to the Biobanco-IMM we reserve the right to reject and discard the specimens, in this case it will be necessary to record the “not conformity” in the Biobanco-IMM database.

ii) Registration of DNA tubes in the Biobanco-IMM database; data of each specimen must be recorded in electronic and paper archive.

iii) The DNA tubes should be immediately processed or, if processing is not possible in the 24h after the arrival of the sample, stored at –80 °C (it is recommended to store a blood sample as backup and to record storage details in the Biobanco-IMM database).

iv) *DNA isolation*: DNA isolation must be performed by appropriate kits that guarantee good yield and integrity of purified DNA.

DNA isolation can be carried out by manual procedure or by using an automated procedure employing robotic workstations. DNA purification is performed according to the manufacture’s protocol.

Researchers, which send to the Biobanco-IMM purified DNA, must declare the DNA extraction procedure, the date of the extraction, the temperature of storage of these samples and the concentration of the DNA..

v) *DNA quantity and quality controls*: DNA concentration is determined after the DNA extraction protocol in the eluted solution, measured by absorbance at 260 nm. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer.

Measure the absorbance at 260 nm and 280nm.

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DNA purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280nm. Pure DNA has an A260/A280 ratio between 1.7-1.9, indicating that the DNA is free from protein contamination.

vi) Take note of sample processing details in the Biobanco-IMM database.

D. Long term storage of DNA samples

After the DNA purification and quantity and quality control, purified DNA must be aliquoted in a 1.2ul cryovials and preserved in –80 °C freezer.

The storage details must be recorded in the Biobanco-IMM database.

E. Backup

It is recommended to split stored biospecimens into two sets of aliquots, each set stored in a different location; this strategy will avoid loss in case of adverse events in one location.

F. Transport of frozen samples

Frozen DNA samples must be transported at -80 °C; it's critical to maintain stable cooling conditions at all times during transport and storage.

The tubes must be transported upright and secured in a leak-proof secondary receptacle. Cushion or suspend tubes during transport. There should be sufficient adsorbent paper around tubes to soak up all liquid in case of a spill. Finally, there should be an outer packaging of adequate strength for its capacity. Dry ice should be placed around the secondary packaging or alternatively in an overpack with one or more complete packages.

The external container should have clearly legible: the name, phone number and address of the Biobanco-IMM; the contact person and; the receiver. , The label "human biological material, handle with care"; the biohazard symbol and the presence of cryogenic gas should be visible in the external container.

Special requirements should be provided to couriers responsible for the transport of the specimens.

Enclose a form containing: number and type of samples with their unique identification codes, diagnosis of donor; date and details of production; date of shipped; temperature of transport; expiry date; notes; instructions for the opening of the packaging and the sample container and information about the presence of cryogenic gas.

The receiving laboratory must be informed about the shipment and probable date of arrival of the samples. Once arrived, the receiving laboratory should communicate to the Biobanco-IMM the arrival of the samples.

The transport details must be recorded in the Biobanco-IMM database

G. Stability of the purified DNA

The stability of the purified DNA is not determined. However, it is expected that DNA samples remain stable over a period of 10 years.

5. RECORDS

Records' Identification	Indexation	Archive Responsible
FORM.BIO.001	Base de dados LIMS	Ângela Afonso
Questionares	Base de dados LIMS	Ângela Afonso

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6. INFORMATION

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B. Documentation:

- **SOP** – SOP.BIO.002