



JAN 09, 2024

L-3 LEECH STORAGE

REDI-NET
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DISCLAIMER

This work is supported by the US Army Medical Research and Development Command under Contract No.W81XWH-21-C-0001, W81XWH-22-C-0093 and HT9425-23-C-0059. The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army or Navy position, policy or decision unless so designated by other documentation.

ABSTRACT

This protocol describes leech storage.

OPEN  ACCESS



DOI:
dx.doi.org/10.17504/protocols.io.rm7vzx234gx1/v1

Protocol Citation: REDI-NET Consortium 2024. L-3 LEECH STORAGE. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.rm7vzx234gx1/v1>

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Protocol status: Working
We use this protocol and it's working

Created: Nov 17, 2023

Last Modified: Jan 09, 2024

PROTOCOL integer ID:
91102

Keywords: STORAGE
PROCEDURE FOR
UNTREATED SAMPLE,
STORAGE PROCEDURE FOR
TOTAL NUCLEIC ACID

Funders

Acknowledgement:

USAMRAA
Grant ID: W81XWH-21-C-
0001

USAMRAA
Grant ID: W81XWH-22-C-
0093

USAMRAA
Grant ID: HT9425-23-C-0059

GUIDELINES

OBJECTIVE

To outline steps for properly storing field-collected leech samples and nucleic acid samples purified from these soil.

SUMMARY/SCOPE

The overarching aim of the REDI-NET is to develop a collaborative laboratory network between domestic and international partnering institutions to address disease surveillance needs in order to effectively detect, predict and contain potentially emergent zoonosis. This SOP provides guidance on storage of leech samples and their purified nucleic acid to preserve their integrity for downstream nucleic acid extraction and/or sequencing library preparation.

MAINTENANCE OF EQUIPMENT

Decontaminate a PCR workstation by keeping the UV light on for  00:15:00 .

MATERIALS

EQUIPMENT AND MATERIALS

Note

NOTE: If product number is listed, please ensure use of this or equivalent product.

A	B	C
Equipment / Material	Description	Mfg / Product #
–80°C freezer	For sample storage	Locally sourced
Forceps	Clean, stainless	Locally sourced
Ice	To maintain cold chain during sample handling	Locally sourced
96-Well Microfuge tube racks with cover	To hold microplates	Locally sourced
KingFisher™ 96 KF microplate	To store the sample	ThermoFisher, 97002540
PCR Workstation	PCR cabinet with UV light	Locally sourced
Clear Adhesive Film	To seal the KingFisher™ 96 KF microplate	ThermoFisher, 4306311
Adjustable micropipettes	To handle the samples	Locally sourced
Multi-channel micropipettes	8- or 12- channel; to handle the sample	Locally sourced
Nuclease-free filter tips low-retention	To ensure appropriate sample handling	Locally sourced
Nuclease free microfuge tubes	1.5 mL	Locally sourced
Saran wrap	Plastic wrap; to seal rack holding sample	Locally sourced
Permanent markers	To label tubes and microplates	Locally sourced
Data sheet	REDI-NET DCS T-3 Tick Storage	REDI-NET Data Portal

SAFETY WARNINGS



RISKS AND PERSONAL PROTECTION:

Gloves should be worn all the time when handling samples.

STORAGE PROCEDURE FOR UNTREATED SAMPLE

1

Note

NOTES:

- Collected leeches need to be kept on cold chain all the time to prevent death and RNA degradation. Viable leeches can be temporarily stored in water at 4 °C before being stored individually in -80 °C freezer. Store leeches at -80. °C until sample processing.
- If -80 °C storage is not possible, temporarily store the leech samples in a -20 °C freezer and follow leech sample processing SOP (REDI-NET Leech Processing SOP L-2) as soon as possible for total nucleic acid extraction. Subsequently, use a portion of the total nucleic acid and reverse- transcribe RNA into cDNA for -20 °C storage. To do this, follow the initial steps of the leech sample testing SOP (REDI-NET Leech Testing SOP L-4) cDNA Synthesis until finishing step 40.

Cool 96-well microfuge tube racks On ice .

2



Label 2.0 mL microfuge tubes with Sample ID.

3

Using clean forceps, transfer individual leech into the corresponding pre-labeled 2.0 mL microfuge tubes and put it onto the microfuge tube rack On ice sequentially.

4

Once the rack is full or all leech samples have been completed, label the rack ID with a unique ID.

- 5 Close the rack lid tightly, secure with clear Saran wrap and immediately transfer to  -80 °C or  -20 °C freezer.

Note

NOTES:

- If -20°C, total nucleic acid extraction needs to be performed preferably within 3 days.

- 6 Update the freezer inventory so samples can be tracked properly.


STORAGE PROCEDURE FOR TOTAL NUCLEIC ACID

7



Note

NOTES:


- The following procedure is to properly store total nucleic acid extracted from leech samples (including negative controls) using the KingFisher nucleic acid purification system. The eluted total nucleic acid will be in either 96-well microplate (Flex model) or elution strip (Duo Prime model).
- Total nucleic acid samples need to be kept  On ice all the time to minimize RNA degradation.

In the clean PCR workstation, carefully transfer the eluted total nucleic acid to a 96-well PCR microplate, make sure to keep samples in the exact same locations corresponding to the rack where the original ticks were stored.

Note

IMPORTANT: Mark the “A1” position of the 96-well microplate to prevent any mistakes on plate orientation.

- 8 Cover the 96-well PCR microplate with adhesive film to prevent spill over or contamination.

- 9 Label the film with a unique plate ID.
- 10 Immediately transfer the 96-well PCR microplate to  -80 °C freezer.
- 11 Update the freezer inventory so that samples can be tracked properly.