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L-3 LEECH STORAGE V.1

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Remote Emerging Disea...

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We use this protocol and it's working

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Disclaimer

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Abstract

This protocol describes leech storage.

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

Troubleshooting

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.

Protocol references

REFERENCES

REDI-NET Overview Summary