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REDI-NET FF-3 FILTH FLY STORAGE

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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

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 filth fly samples and their purified nucleic acid to preserve their integrity for downstream nucleic acid extraction and/or sequencing library preparation.

Guidelines

To outline steps for properly storing filth fly samples and nucleic acid samples purified from these samples.

Materials

	A	B	C
	Equipment / Material	Description	Mfg / Product #
	–80°C freezer	For sample storage	Locally sourced
	Ice	To maintain cold chain during sample handling	Locally sourced
	96-Well Microfuge tube racks with cover	To hold the samples	Locally sourced
	KingFisher‰ 96 KF microplate	To store the TNA samples	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

Safety warnings

RISKS AND PERSONAL PROTECTION:

Gloves should be worn all the time when handling samples.

Before start

MAINTENANCE OF EQUIPMENT:

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

STORAGE PROCEDURE FOR UNTREATED SAMPLE

1 Precool 96-well microfuge tube racks on ice.

Note

NOTE: Filth fly samples need to be kept on a cold chain all the time to prevent RNA degradation. The following procedure will apply only where -80°C storage is feasible and flies are not being kept alive for research purposes.

NOTE: If -80 °C storage is not possible, temporarily store the filth fly samples in a -20 °C freezer and follow filth fly sample processing SOP (REDI-NET SOP FF-2 Filth Fly Processing) 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 filth fly sample testing SOP (REDI-NET SOP FF-4 Filth Fly Testing) until finishing the step of “Removal of RNA”.

2 Using preprinted adhesive labels or permanent markers, label 1.5 mL microfuge tubes with unique sample ID.

3 Transfer filth sly sample into the corresponding pre-labeled 1.5 mL microfuge tubes, close the cap and put it onto the microfuge tube rack (on ice) sequentially.

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

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

STORAGE PROCEDURE FOR TOTAL NUCLEIC ACID

6

Note

NOTE: The following procedure is to properly store total nucleic acid extracted from filth fly samples (including negative controls) using 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).

NOTE: Total nucleic acid samples need to be kept on ice all the time and as short as possible to minimize RNA degradation.

In the clean PCR workstation, carefully transfer the eluted total nucleic acid to a 96-well PCR microplate, making sure to keep samples in the exact same locations (e.g., A1 to A1, A2 to A2...) corresponding to the rack where the original samples were stored. IMPORTANT: Mark the “A1” position on the 96-well microplate to avoid any mistakes on plate orientation.

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

8 Label the film with a unique plate ID.

9 Immediately transfer the 96-well PCR microplate to -80 °C freezer.

Protocol references

REDI-NET Overview