



JAN 09, 2024

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**DOI:**  
[dx.doi.org/10.17504/protocols.io.j8nlko241v5r/v1](https://dx.doi.org/10.17504/protocols.io.j8nlko241v5r/v1)

**Protocol Citation:** REDI-NET Consortium 2024. S-3 SOIL STORAGE. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.j8nlko241v5r/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

## 🌐 S-3 SOIL STORAGE

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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 soil storage.

**PROTOCOL integer ID:**  
91097

**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 soil 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 soil 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

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### Note

#### NOTES:

- Collected soil samples need to be kept on a cold chain at all times to prevent RNA degradation. The following procedure will apply only where -80 °C storage is feasible.
- If -80 °C storage is not possible, temporarily store the soil samples in a -20 °C freezer and follow soil sample processing SOP (REDI-NET SOP S-2 Soil 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 soil sample testing SOP (REDI-NET SOP S-4 Soil Testing) cDNA Synthesis until finishing step 40.

Cool 96-well microfuge tube racks On ice .

- 2 Each collected soil sample will be transferred to a 50 mL falcon tube with a unique sample ID. Put falcon tubes into cryo/freezer box.
- 3 Label the boxes with unique box ID.
- 4 Close the box tightly, secure with clear Saran wrap and immediately transfer to -80 °C freezer.
- 5 Update the freezer inventory so that samples can be tracked properly.


## STORAGE PROCEDURE FOR TOTAL NUCLEIC ACID

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### Note

#### NOTES:

- The following procedure is to properly store total nucleic acid extracted from soil 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.

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.

10 Update the freezer inventory so that samples can be tracked properly.

