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RNA Purification from Buccal Swabs, Nasopharyngeal Samples (swab or aspirate) and Saliva using the Monarch Total RNA Miniprep Kit V.1

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Abstract

This protocol utilizes the Monarch Total RNA Miniprep Kit to purify RNA from buccal swabs, nasopharyngeal samples, and saliva.

Guidelines

This protocol is to be used for research use only.

Materials

MATERIALS

Nuclease-free Water

Microcentrifuge

Monarch Total RNA Miniprep Kit **New England Biolabs Catalog #**T2010S

RNase-free Microfuge Tubes (0.5 mL) **Thermo Fisher Catalog #**AM12300

RNase-free Microfuge Tubes (1.5 mL) **Thermo Fisher Catalog #**AM12400

Additional Materials:

- isopropanol
- ≥95% ethanol
- 2X Monarch DNA/RNA Protection Reagent
- collection tubes (additional)
- Monarch Proteinase K
- Monarch Lysis Buffer
- 1X Monarch DNA/RNA Protection Reagent

Troubleshooting

Safety warnings

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Before start

- Monarch DNA/RNA Protection Reagent is supplied as a 2X concentrate. Dilute with nuclease-free water only as needed, as some sample types require resuspension in the 2X concentrate, while others require a 1X solution. If purifying samples stored in Monarch DNA/RNA Protection Reagent, please review the related guidance.
- For the 50 prep kit, add 275 µl nuclease-free water to the lyophilized DNase I vial and resuspend by gentle inversion. We suggest making aliquots of DNase I, sized to your processing needs, and storing at -20°C to minimize freeze-thaw cycles (3 F/T cycles maximum)
- For the 50 prep kit, add 1,040 µl Proteinase K Resuspension Buffer to the lyophilized Proteinase K (Prot K) vial and vortex to resuspend. Store at -20°C.
- For the 50 prep kit, add 100 ml ethanol ≥ 95% (not included) to the 25 ml RNA Wash Buffer concentrate and store at room temperature
- Addition of RNA Lysis Buffer and all subsequent steps should be performed at room temperature (this will prevent precipitation of detergent in the lysis buffer). If samples are accidentally placed on ice and precipitate forms, allow the samples to return to room temperature to resolubilize before loading onto the column.

1 Sample Disruption and Homogenization

STEP CASE

Buccal + nasopharyngeal with transport medium 1 step

Buccal swabs and nasopharyngeal samples with transport medium

2 Place swab into a tube containing 300 µL of 1X Monarch DNA/RNA Protection Reagent to an aliquot of transport medium and vortex briefly.

3 For every 300 µL of DNA/RNA Protection Reagent/Sample Mixture , add 15 µL Monarch Proteinase K.

4 Vortex briefly and incubate at Room temperature for 00:30:00 .

5 Vortex sample briefly and spin for 00:02:00 (16,000 x g) to pellet debris.

6 Transfer supernatant to an RNase-free microfuge tube.

7 Add an equal volume of **Monarch RNA Lysis Buffer** and vortex briefly.

8 Proceed to **Step 1** of **Part 2: RNA Binding and Elution**

Part 2: RNA Binding and Elution

9

Note

All centrifugation steps should be carried out at 16,000 x g.

Transfer up to 800 µL of the sample from Part 1 to a gDNA Removal Column (light blue) fitted with a collection tube.

Note

For sample identification, label collection tubes, as gDNA removal columns will be discarded after spinning.

10 Spin for 00:00:30 to remove most of the gDNA.

Note

SAVE THE FLOW-THROUGH (RNA partitions here).

11 Discard the gDNA Removal Column.

12 Add an equal volume of ethanol (≥ 95%) to the flow-through and mix throughly by pipetting.

Note

To exclude RNA ≤ 200 nt, add only 1/2 volume ethanol to flow-through. The addition of ethanol creates favorable conditions for RNA to bind to the RNA Purification column.

13 Transfer mixture to an RNA Purification Column (dark blue) fitted with a collection tube.

14 Spin for 00:00:30 .

15 Discard flow-through.

Note

If further gDNA removal is essential for downstream applications, proceed to on-column DNase I treatment, Step 10.1–10.3 (recommended). If not, proceed to Step 5.

15.1 Add 500 µL RNA Wash Buffer and spin for 00:00:30 and discard flow-through.

Note

This ensures all salts are removed prior to the addition of DNase I.

Note

If using a vacuum manifold, add 500 µl of RNA Wash Buffer and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.

15.2 In an RNase-free microfuge tube (not included), combine 5 µL DNase I with 75 µL DNase I Reaction Buffer r and pipet mixture directly to the top of the matrix.

15.3 Incubate for 00:15:00 at Room temperature .

16 Add 500 µL RNA Priming Buffer and spin for 00:00:30 .

17 Discard flow-through.

Note

If using a vacuum manifold, add 500 µl of RNA Priming Buffer and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.

18 Add 500 µL RNA Wash Buffer and spin for 00:00:30 .

19 Discard flow-through.

Note

If using a vacuum manifold, add 500 µl of RNA Priming Buffer and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.

20 Add another 500 µL RNA Wash Buffer and spin for 00:02:00 .

21 Transfer column to an RNase-free microfuge tube.

Note

Use care to ensure the tip of the column does not contact the flow-through. If in doubt, re-spin for 1 minute to ensure no ethanol is carried over.

Note

If using a vacuum manifold, add 500 µl of RNA Wash Buffer and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.

22 Add 30 µL to 100 µL Nuclease-free Water directly to the center of the column matrix and spin for 00:00:30 .

Note

For best results, elute with at least 50 µl, which is the minimum volume needed to wet the membrane. Lower volumes can be used but will result in lower recovery (elution in 30 µl results in > 80% recovery and 100 µl provides maximum recovery). For spectrophotometric analysis of eluted RNA, it may be necessary to re-spin eluted samples and pipet aliquot from top of the liquid to ensure that the A 260/230 is unaffected by possible elution of silica particles.

23 Place RNA on ice if being used for downstream steps at:

-20 °C short-term storage (less than one week)

-80 °C long-term storage .

Note

Addition of EDTA to 0.1–1.0 mM may reduce the activity of any contaminating RNases.