NUCLEIC ACID EXTRACTION KIT

Cat. No.: MDXC002

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COLUMN BASED EXTRACTION KIT-
20 TESTS

1 Contents

CONTENT	QUANTITY	STORAGE
Column Lysis Buffer	12 mL	Room Temperature
Tissue Lysis Buffer	8 mL	Room Temperature
Reaction Tube	20 Tubes	Room Temperature
Wash Buffer RE	10 mL	Room Temperature
Wash Buffer RW	5 mL	Room Temperature
Homogenizer	1 Number	Room Temperature
Elution Buffer	1 mL	Room Temperature

Items Required by The User But Not Provided In The Kit –

1. RNase/DNase-free plastic ware including 1.5ml micro-centrifuge tubes, aerosol resistant barrier tips
2. Table top micro-centrifuge (with rotor for 1.5ml and 2ml tubes).
3. Vortex mixer
4. Pipettes – Variable volume
5. Water bath
6. 100% Ethanol

2 Storage and Stability

The Nucleic Acid Extraction Kit components must be stored at room temperature. Kit components are guaranteed to be stable thr ough the expiration date printed on the label.

⚠ Please note, that improper storage at +2 to +8°C (refrigerator) or –15 to –25°C (freezer) will adversely impact nucleic acid purification when precipitates form in the solutions.

3 Application

The Nucleic Acid Extraction Kit is designed for the purification of RNA/DNA from Nasopharyngeal swab, Throat swab, Viral Transport media, Blood fluids, serum plasma, Bacterial and viral cell culture, urine, Tissue samples sample etc.

Up to 20 samples can be processed simultaneously in approx. 1 hour. Thus, the purification procedure is less time consuming compared with alternative methods which require extraction with organic solutions, RNA/ DNA precipitation or ultracentrifugation.

4 General Considerations Handling Requirements

⚠ **Lysis Buffer and Wash Buffer RE contain guanidine hydrochloride which is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling.**

⚠ **Avoid contact of the Lysis Buffer and Wash Buffer RE with the skin, eyes, or mucous membranes. If contact does occur, immediately wash the affected area with large amount of water. Burns can occur if left untreated. If the reagent spills, dilute with water before wiping dry.**

⚠ **Do not use any modified ethanol.**

⚠ **Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions. After first opening, store all bottles in an upright position.**

5 Safety Information

Laboratory Procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, mask, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipettes and nuclease-free pipette tips only, to remove aliquots from reagent bottles.

6 Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on www.ubio.com, or upon request from office.

7 Protocol

1. Add 600µL Column Lysis Buffer into a Reaction Tube.
2. Mix it well to resuspend the dried reagent.
3. Add 200µl of sample (Nasopharyngeal swab, Throat swab, Viral Transport media, Blood fluids, serum plasma, Bacterial and viral cell culture, urine, Tissue samples) to the above tube.
4. Pulse-vortex the sample for about 15-30 secs in a vortex mixer and incubate the samples at 72°C for 10 min.
5. After incubation add 450µl absolute ethanol and mix by pulse vertexing.
6. Briefly centrifuge the tube to remove drops from the lid.
7. Transfer 600µl mix into column placed in 2ml collection tube.
8. Centrifuge at 11,000 RPM for 5 min.
9. Discard the flow through in collection tube.
10. Add the remaining mix to the column and repeat the steps 8 and 9.
11. Add 500µL wash buffer RE and centrifuge at 11,000 RPM for 2 min. Discard the flow through.
12. Add 500µL wash buffer RW, centrifuge at 11,000 RPM for 2 min.
13. Discard the flow through.
14. Repeat step 12 and 13 with 500µl wash buffer RW.
15. After discarding place, the binding column back into the same collection tube. Centrifuge the empty column at 12,000 RPM for 5 min to completely remove ethanol.
16. Place column to a new RNase/DNase free centrifuge tube and add 50µl of pre-heated (60°C) Elution buffer to the center of the column.
17. Incubate at room temperature for 1 min and centrifuge at 11,000 RPM for 1min.
18. Collect the elute to a fresh RNase/DNase free Eppendorf tube.Note: Store eluted sample at -20°C to -80°C.

Note: **Pretreatment for tissue samples**

- Add 400µL of Tissue Lysis Buffer to 20 - 50 mg of tissue.
- Homogenize the tissue sample using homogenizer.
- Centrifuge the homogenized sample at 8000 RPM for 5 min.
- Collect the supernatant and discard the pellet
- 200 µL of the supernatant use as the sample and follow the protocol mentioned above.

8 Troubleshooting

Observation	Possible cause	Recommendation
Low nucleic acid yield or purity	Kits stored under non-optimal conditions	Store kit at room temperature
	Buffer or other reagents were exposed to conditions reducing their functionality	Store all buffers at +15 to +25^{0}C
		Close all reagent bottles tightly after each use to prevent change in pH, stability. Mix the buffer well and store at room temperature.
	Reagents and samples not completely mixed	Always mix the sample tube properly after addition of each reagent.
Poor elution of nucleic acids with water	Elution buffer has the wrong pH	If you use your own water or buffer to elute nucleic acids from tube, make sure that it has the same pH as the Elution Buffer supplied in the kit
Low RNA/ DNA yield	High levels of RNase/DNase activity	Be careful to create an RNase/DNase-free working environment.
		Process sample immediately or store it at –80°C until it can be processed.
		Use eluted RNA/DNA directly in downstream procedures or store it immediately at –80°C