CE IVDR

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AZUL STOOL RNA EXTRACTION KIT

RNA IN 75 MINS | GOOD YIELDS FOR USE IN PCR/SEQUENCING

PRODUCT BROCHURE

Cat No-RE108

ISO 13485 CERTIFIED

PRODUCT DESCRIPTION

AZUL Stool RNA Extraction Kit is an easy and efficient system for the isolation of high-quality RNA (human and bacterial) from fresh, frozen stool or from stool samples stored in stabilization solution. This kit uses a silica-based spin column technology for isolating RNA from biological samples, thereby eliminating toxic phenol-chloroform extractions. The eluted RNA is suitable for all sensitive downstream applications such as qPCR and Next-Generation sequencing.

KIT COMPONENTS

Components	For 50 preps	For 25 preps
Extraction Buffer	35 mL	20 mL
Lysis Buffer 1 (LB1)	2 mL	1 mL
Lysis Buffer 2 (LB2)	1 mL	0.5 mL
Stabilization Buffer (SB)	50 mL	25 mL
Proteinase K	1.3 mL	750 µL
Binding Buffer (BB)	30 mL	15 mL
Wash Buffer 1 (WB1)	30 mL	15 mL
Wash Buffer 2 (WB2)	25 mL	15 mL
Elution Buffer (EB)	4 mL	2 mL
Spin Column	50 (Pouch pack)	25 (Pouch pack)

SPECIFICATIONS

Format	Spin Column
Sample type	Fresh, Frozen stool samples, Stool samples stored in stabilization solution.
Equipment	Microcentrifuge
Processing time	75 Mins
Sample amount	>200 µL - Liquid/stored stool samples >200mg - Solid samples
Type	Total RNA
Sample storage	Eluted RNA should be stored at ≤ -20°C
Yield	25 - 50 µg
Purity	A260/280 ≥ 1.8 - 2
Kit Storage	Room Temperature
Kit Validity	Viable for 1 year if stored at appropriate conditions

NOTE: *Check the Extraction Buffer, Binding Buffer and Lysis Buffer for any salt precipitation before every use. Re-dissolve any precipitate by warming the solution to 37°C, then cool it back to room temperature before use.*

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RNA EXTRACTION PROTOCOL

1. Collect and weigh >200 mg of fresh, frozen stool samples or transfer >200 µL stool samples stored in stabilization solution to a clean microfuge tube and add 1 mL of Stabilization Buffer (SB).
2. Centrifuge the tube at 10,000 rpm for 5 mins.
3. Discard the supernatant, to the pellet add 500 µL-700 µL of Extraction Buffer (EB), and 25 µL of Lysis Buffer 1 (LB1).
4. Mix briefly by vortexing for 30 secs.
5. Add 20 µL of Lysis Buffer 2 (LB2) to the mixture and mix well. Incubate the tubes at 37°C for 10-15 mins or until the solution turns clear.
6. Add 25 µL of Proteinase K to the tube and incubate at 56°C for 10 mins with intermittent vortexing every 5 mins.
7. Centrifuge the tube at 15,000 rpm for 15 mins at RT. Transfer the clear supernatant to a new microfuge tube.
8. Add 600 µL Binding Buffer (BB) to this suspension and mix briefly by inverting the tube a few times.
9. Transfer the suspension to a spin column and centrifuge the tube at 15,000 rpm for 2 mins at RT.
10. Discard the flow-through and place the purification column back into the collection tube. Repeat this step until complete lysate has been transferred into the column and centrifuged.
11. Wash the spin column with 600 µL Wash Buffer 1 (WB1) at 15,000 rpm for 1 min and discard the flow through.
12. Add 500 µL of Wash Buffer 2 (WB2) to the column and centrifuge at 15,000 rpm for 1 min to completely remove salts and impurities.
13. Keep the purification column in a clean, sterile 1.5 mL microfuge tube and add 30 µL-50 µL of Elution Buffer or DNase/RNase-free water to the center of the column.
14. Centrifuge the column for 15,000 rpm for 2 mins.
15. Discard the purification column and store the eluted RNA at -20°C or -80°C until use.

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FLOW DIAGRAM OF RNA EXTRACTION PROTOCOL

Weigh ≥200 mg of dry sample
>200µL of Liquid/stored stool sample
+
1 ml of Stabilization solution

Centrifuge at 10,000 rpm for 5 mins

To the pellet add 500-700µL Extraction Buffer, 25µL Lysis Buffer 1 and 20µL Lysis Buffer 2

Incubate at 37°C for 10-15 mins

25µL Proteinase K
Incubate at 56°C for 10mins

Centrifuge at 15,000 rpm for 15 mins

Transfer clear supernatant to new tube

Add 600 µL
Binding Buffer

Mix well
Transfer lysate to Spin column

Centrifuge at 15,000 rpm for 2 mins

Add 600 µL Wash Buffer 1

Centrifuge at 15,000 rpm for 1 min

Add 500 µL Wash Buffer 2

Centrifuge at 15,000 rpm for 1 min

Add 30-50 µL Elution Buffer

Centrifuge at 15,000 rpm for 2 mins

Eluted RNA

Store at -20°C or
-80°C until use

TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSES	SUGGESTED SOLUTIONS
Low RNA Yield	**Sample input:** Too much sample input or significantly less sample used	Use less input material or increase the volume of the Extraction Buffer for better lysis. Use of ≥ 200 mg or >200 µL of sample is recommended for good RNA yield.
	Incomplete Debris Removal or incomplete lysis/homogenization can cause debris to clog or overload the column and leech salts into RNA eluate.	Increase the volume of extraction Buffer to ensure complete homogenization. Be sure to centrifuge and pellet any debris and transfer the supernatant while avoiding any pellet debris.
Low RNA Purity(A260/A280)	Improper sample handling results in ethanol or salt contamination	Make sure lysate and wash buffers have passed entirely through the matrix of the column. This may require centrifuging at a higher speed or longer time.
DNA Contamination	Too much sample used	**To remove DNA:** Perform in-column DNase I treatment or perform DNase I treatment post-purification (not provided in the kit), then re-purify the treated sample.
RNA Degradation	Use of old samples	**To prevent RNA degradation:** Immediately collect and lyse fresh stool samples into a Extraction Buffer. Collect and store the fresh stool samples in mWRAPR Fecal RNA Solution to ensure stability & integrity of RNA and process later.

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ORDERING INFO

CATALOG NO	PRODUCT	PREP
RE101	AZUL SARS- CoV-2 Kit RNA Extraction Kit	25/50 preps
RE102	AZUL Tissue RNA Extraction Kit	25/50 preps
RE103	AZUL Bacterial RNA Extraction Kit	25/50 preps
RE104	AZUL Plant RNA Extraction Kit	25/50 preps
RE105	AZUL Soil RNA Extraction Kit	25/50 preps
RE106	AZUL Animal Cell Culture RNA Extraction Kit	25/50 preps
RE107	AZUL Blood RNA Extraction Kit	25/50 preps
RE108	AZUL Stool RNA Extraction Kit	25/50 preps
RE109	AZUL Saliva RNA Extraction Kit	25/50 preps
RE113	AZUL Microbiome RNA Extraction Kit	25/50 preps
RE114	AZUL Fungal RNA Extraction Kit	25/50 preps
RE115	AZUL FFPE RNA Extraction Kit	25/50 preps

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FEEDBACK

How did this kit perform?

Did AZUL Extraction Kit fulfill expectations required for your research?
Let us know by filling out the feedback form here

Or scan the QR code:

CONTACT US

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