EX-RNA™ - SPINTUBE-CR

RNA Extraction Kit with Carrier RNA For RT-PCR Testing (SPINTUBE METHOD)

(For *in vitro* diagnostic use only)

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INTENDED USE

This kit is recommended to be used for the isolation of viral RNA from various samples like fresh and frozen plasma, serum, nasopharyngeal swab, oropharyngeal swab, sputum, bronco-alveolar lavage in viral transport medium and other body fluids. The system is intended for use by professional users trained in molecular biological techniques.

SUMMARY

EX-RNA™-SPINTUBE-CR is a simple and efficient system for extraction and purification of total RNA from samples like fresh and frozen plasma, serum, nasopharyngeal swab, oropharyngeal swab, sputum, bronco-alveolar lavage in viral transport medium and other body fluids. Ribonucleic acid (RNA) purified from biological materials is utilized extensively for molecular biology research and is becoming an important tool in human clinical testing.

PRINCIPLE

Samples are lysed and homogenized in lysis buffer, which contains chaotropic salts. Chaotropic salts have two important roles in nucleic acid extraction. Firstly, they destabilize hydrogen bonds, van der Waals forces and hydrophobic interactions, leading to destabilization of proteins, including nucleases. Secondly, they disrupt the association of nucleic acids with water, thereby providing optimal conditions for binding. Buffering conditions enhances the binding of nucleic acids in the column. After centrifuging the lysate through the silica membrane, the desired nucleic acids are bound to the column and unbound components are in the flow-through. However, the membrane will contain impurities such as protein, polysaccharides and salt residues. The wash steps remove such impurities. Subsequent addition of elution buffer to the column will hydrate the nucleic acids which is collected in the elute collection tube.

PRESENTATION

REF	1108150100
	100 Tests

Kit Components (for 100 Tests):

Reagents:

R1: Lysis Buffer	60 mL
R2: Wash Buffer	30 mL
R3: Elution Buffer	10 mL
CR: Carrier RNA (Poly A)	500 µg

Accessories:

T1: Sample Lysis Tubes	100 Nos.
T2: Spin Column with Wash Tubes	100 Nos.
T3: Elute Collection Tubes	100 Nos.
Packinsert	1 No.

STORAGE AND STABILITY OF THE KIT

The kit and reagents are stored at 20-25°C. Precipitate can be seen in the Lysis Buffer when the buffer is stored at low temperature as it contains high concentration of salts. Please heat the buffer at 55°C for 10-15 minutes to completely dissolve the salts before use.

REAGENT/MATERIALS REQUIRED BUT NOT PROVIDED

1. Ethanol (96-100%)
2. RNase inactivating agents such as **DECON-R™** (10 x 200 mL - Cat. No.1108092000)
3. Micro-centrifuge (operational requirement 2500 x g - 6000 x g)
4. Vortex
5. Microtube Stand
6. Sterile Nuclease free microtips
7. Micropipettes

WARNINGS

EX- RNA™-SPINTUBE-CR contains guanidine thiocyanate which is corrosive to metals, causes skin corrosion and serious eye damage.

- Recommended Personal Protective Equipment Includes Dust mask type N95, Eye shields and thick durable Nitrile or Plastic Gloves .
- If on skin: Gently wash with plenty of water.
- If skin irritation or rash occurs: Get medical advice/attention.

Kindly note that this transport media should not be used in a testing platform that uses bleach or in laboratories that use bleach as a part of their routine decontamination and disposal process. When the bleach interacts with guanidine thiocynate it produces the hazardous cyanide gas.

PRECAUTIONS

1. A thorough understanding of the pack insert is mandatory before performing the test for the first time. Adherence to protocol specified herein is necessary to ensure optimal performance of the product. Any deviation from the assay procedure may affect the results.
2. Do not mix reagents of different lots.
3. Do not use reagents beyond their expiry date.
4. Use nuclease free plasticware and water.
5. Avoid any contamination among samples; for this purpose, disposable tips should be used for each sample and reagent.
6. Do not use reagents from other manufacturers along with the kit reagents for a given test run.
7. Do not interchange reagent vials and their screw caps to avoid cross contamination. Use a clean, fresh, disposable pipette tip for each reagent or specimen manipulation.
8. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
9. Always wear gloves. After wearing gloves, do not touch surfaces and equipment to avoid reintroduction of RNases to material.
10. Treat surfaces of benches and glassware with RNASE inactivating agents such as DECON-R™ (10 x 200 mL - Cat No.1108092000).
11. Use sterile, disposable RNase-free plastic ware.
12. Use only DEPC-treated water or nuclease free water such as RNASE FREE WATER™ (10 x 500 mL - Cat. No.1108115000).
13. Best results were obtained by using fresh samples or samples that have been quickly frozen in liquid nitrogen or stored at -70°C.
14. Practice Good Laboratory practice (GLP) while handling specimen and other reagents.
15. Follow precautions as per the handling of specimen capable of transmitting infectious agents.
16. Lysis buffer contains chaotropic salt. It is not compatible with disinfecting agents that contain bleach. Dispose it as per local medical waste guidelines.

PREPARATION OF REAGENTS:

Reagents provided as a concentrate

Reagents	Contents/Labels	Volume of Ethanol (96 -100 %) to be added
R2	Wash Buffer	30 mL Ethanol

Carrier RNA (Poly A): Carrier RNA (Poly A) is provided in lyophilized format. Add 550 µL of R3: Elution Buffer to the tube. Dissolve the Carrier RNA (Poly A) thoroughly, divide it into conveniently sized aliquots and store it at -30 to -15 °C. Do not freeze-thaw the aliquots more than 3 times.

Reagents Not Provided, to be prepared by the User

Reagents	Contents/Labels	Volume of Ethanol (96 -100 %) to be added
B1	Binding Reagent	60 mL Ethanol
W2	Ethanol Wash Solution	45 mL Ethanol + 15 mL Nuclease free water

Note: R2 is supplied as concentrate. Carrier RNA (Poly A) is provided in lyophilized format. **B1** and **W2** to be prepared separately by user. Before using for the first time, add recommended volume of ethanol (96–100%) as indicated in the table above and also label them on the respective bottles. Please tick mark the check box provided on the label after addition of ethanol.

PROCEDURE:

1. Take 500 µL of R1, Lysis Buffer in T1, Sample Lysis Tube. Add 5 µL of Carrier RNA (Poly A) and mix thoroughly by pipetting. Then Add 300 µL of sample to it.
2. Add 500 µL of B1, Binding Reagent and with the same tip mix well the reaction mix by pipetting up and down 10 to 15 times and incubate for 10 minutes at room temperature.
3. Transfer 650 µL of this suspension into T2: Spin Column with Wash Tube.
4. Centrifuge at 6000 x g for 1 minute at room temperature. Discard the flow-through from wash tube and blot dry on absorbent paper.
5. Add the remaining 650 µL suspension from the T1: Sample Lysis Tube into the same T2: Spin Column with Wash Tube.
6. Centrifuge at 6000 x g for 1 minute at room temperature. Discard the flow-through from wash tube and blot dry on absorbent paper.
7. Transfer 500 µL of prepared reagent R2, Wash Buffer in the T2: Spin Column with Wash Tube.
8. Centrifuge at 6000 x g for 1 minute at room temperature. Discard the flow-through from wash tube and blot dry on absorbent paper.
9. Transfer 500 µL of prepared reagent W2, Ethanol Wash Solution in the T2: Spin Column with Wash Tubes.
10. Centrifuge at 6000 x g for 3 minutes at room temperature. Discard the flow-through from wash tube and blot dry on absorbent paper.
11. Dry spin the T2: Spin Column with Wash Tubes at 6000 x g for 3 minutes at room temperature.
12. Retain the spin column, discard the wash tube and transfer the spin column in T3: Elute Collection Tube.
13. Add 70 µL of R3, Elution Buffer and incubate for 10 minutes at 35 °C.
14. Centrifuge at 6000 x g for 3 minutes at room temperature.
15. Elute is collected in elute collection tube. Discard the spin column.
16. Close the lid of elute collection tube and label appropriately.

Storage of the elute with purified RNA: The elute contains pure RNA, recommended to be stored at lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturing of RNA.

PERFORMANCE CHARACTERISTICS

The product is approved by NIV, Pune, an ICMR centre.

Clinical Evaluation

Clinical Evaluation was conducted at Sri Aurobindo Medical College & PG Institute, SAIMS Hospital. Performance comparison of **EX-RNA™ SPINTUBE-CR** was done against an ICMR approved Commercial Extraction Kit and validated on SARS COV 2 RT PCR platform. A total of 65 random samples were taken from the patients clinically suspected for COVID-19 and comparative runs were performed to assess the sensitivity and specificity of the "**EX-RNA™ SPINTUBE-CR**". **Specificity:** 44 negative runs correlated between the method, depicting 100% specificity for the "**EX-RNA™ SPINTUBE-CR**". **Sensitivity:** 21 positive sample runs correlated between the method, depicting 100 % sensitivity for "**EX-RNA™ SPINTUBE-CR**". All positive and negative sample results were in 100 % concordance with the reference ICMR approved assay.

REFERENCES:

(1).Sambrook, J. & Russell, D. Molecular cloning: a laboratory manual. 3rd edn, (Cold Spring Harbor Laboratory, 2001). (2). Noonberg, S. B., Scott, G. K. & Benz, C. C. Effect of pH on RNA degradation during guanidinium extraction. BioTechniques 19, 731-733 (1995). (3). Velikyan, I., Acharya, S., Trifonova, A., Földesi, A. & Chattopadhyaya, J. The pK(a)'s of 2'-hydroxyl group in nucleosides and nucleotides. Journal of the American Chemical Society 123, 2893-2894 (2001). (4). Data on file: Coral Clinical Systems.

Symbol keys

25°C / 20°C Store at 20-25°C	Manufacturer	IVD *In vitro* Diagnostic Medical Device	R1 Lysis Buffer	T1 Sample Lysis Tubes	CR Carrier RNA (Poly A)	
Use by (Last day of stated month)	Consult Instructions for use	LOT Batch Number	R2 Wash Buffer	T2 Spin Column with Wash Tubes	Health hazard	Contains sufficient for <n> tests
Date of Manufacture	REF Catalogue Number	EC REP Authorised Representative in the European Community	R3 Elution Buffer	T3 Elute Collection Tubes	This way up	Corrosive

RNA5/1020/VER-01

Manufactured by:
Coral Clinical Systems
A Division of Tulip Diagnostics (P) Ltd.
PLOT NO. M-46, BLDG. NO. D, PHASE III B, VERNA IND. EST., VERNA, GOA-403 722, INDIA.
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