EX-RNA™ - MAG

RNA Extraction Kit For RT-PCR Testing (Magnetic Beads 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 nasopharyngeal swab, oropharyngeal swab, serum and plasma specimens using magnetic beads. This kit is for *in vitro* diagnosis use.

SUMMARY

EX-RNA™-MAG is a simple and efficient system for extraction and purification of total RNA from samples like nasopharyngeal swab, oropharyngeal swab, serum and plasma using magnetic beads and is utilized extensively for molecular biology research and is becoming an important tool in human clinical testing.

PRINCIPLE

In the first step the specimen is mixed with the Lysis/Binding Buffer and Magnetic beads during which the specimen is lysed and releases substantial amount of RNA from the cells which binds to the magnetic beads. In the next step the RNA bound to the beads are washed with Wash Buffer A and B sequentially to remove the salts and proteins. Finally, the Elution Buffer is added to elute the RNA from the beads, Eluted RNA can be used for RT-PCR and other molecular testing.

PRESENTATION

A. Standard Kit

REF	1108120048
	48 Tests

Kit Components:

R1: Lysis/Binding Buffer: 25 mL
R2: Magnetic Beads: 850 µL
R3: Wash Buffer A : 35 mL
R4: Wash Buffer B : 15 mL
R5: Elution Buffer: 5 ml
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/Binding 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. Magnetic Beads tend to settle with time. It is necessary to mix the vials vigorously or vortex thoroughly so as to obtain a homogeneous mix.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Magnetic Stand- MagStand™ (1 No.-Cat. No. 20221130060) and a Microtube Stand.
2. RNase inactivating agents such as DECON-R™ (10 x 200 ml -Cat. No.1108092000).
3. Incubator at 35°C.
4. Sterile Nuclease free microtips.
5. Micropipettes.
6. Ethanol (96 - 100 %).
7. Sterile nuclease free water.

WARNINGS

EX-RNA™- MAG Lysis/Binding Buffer 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 the Lysis/Binding Buffer 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 commercially available RNase inactivating agents.
11. Use sterile, disposable RNase-free plastic ware.
12. Use only DEPC-treated water or nuclease free water.
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. Tubes and tips should be disposed into a waste bin containing 10% sodium hypochlorite solution to disinfect the consumables.
15. Practice Good Laboratory practice (GLP) while handling specimen and other reagents.
16. Follow precautions as per the handling of specimen capable of transmitting infectious agents.

PREPARATION OF REAGENTS

Reagents	Contents/ Labels	Volume of (96 -100%) Ethanol to be added
R1	Lysis/ Binding Buffer	25 mL
R3	Wash Buffer A	15 mL
R4	Wash Buffer B	35 mL

Note: R1, R3 and R4 are supplied as concentrate. Before using for the first time, add recommended volume of ethanol (96–100%) as indicated above and on the respective bottles. Please tick mark the check box provided on the label after addition of ethanol.

PROCEDURE:

1. Bring the reagents and samples to room temperature before use.
2. Pipette 800 µL R1, Lysis/Binding Buffer in a 2 mL microcentrifuge tube.
3. Add 400 µL sample and 15 µL R2, Magnetic beads, mix well by pipetting up and down10 to 15 times.
4. Place the tubes in a Microtube stand and incubate for 15 minutes at room temperature.
5. Following incubation, place the tube in a Magnetic stand for 2 minutes. It will draw the Magnetic Bead/NA Complex to the side of the tube. Aspirate and discard the supernatant completely using a pipette without disturbing the Magnetic Bead/NA Complex.
6. Transfer the tubes to a normal Microtube stand.
7. Add 800 µL R3, Wash Buffer A to the tube and thoroughly resuspend the beads by pipetting up and down 10 to 15 times.
8. Place the tube in a magnetic stand for 2 minutes to draw the Magnetic Bead/NA Complex to the side of the tube. Aspirate and discard the supernatant completely using a pipette without disturbing the Magnetic Bead/NA Complex.
9. Transfer the tubes to a normal Microtube stand.
10. Add 800 µL R4, Wash Buffer B to the tube and thoroughly resuspend the beads by pipetting up and down 10 to 15 times.
11. Place the tube in a Magnetic stand for 2 minutes to draw the Magnetic Bead/NA Complex to the side of the tube. Aspirate and discard the supernatant completely using a pipette without disturbing the Magnetic Bead/NA Complex.
12. Transfer the tubes to a normal Microtube stand.
13. Allow the Magnetic Bead/NA Complex to air-dry by keeping the lids open for approx. 10 minutes preferably in an incubator at 35°C.
    NOTE: IT IS IMPORTANT THAT THE MAGNETIC BEADS ARE DRIED COMPLETELY BEFORE CONTINUING WITH THE ELUTION STEP.
14. Add 60 µL of R5, Elution Buffer to the tube and carefully **resuspend the beads completely** with the microtip and by pipetting up and down 10 to 15 times.
15. Incubate the suspension at 35 °C for 10 minutes.
16. Place the tube in a Magnetic stand for 2 minutes to draw the Magnetic Beads to the side of the tube. Aspirate and collect the Elute (~50 to 60 µL) completely using a pipette without disturbing the Magnetic Beads.
17. Transfer the elute containing the purified viral RNA to a clean elute collection tube.

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.

REFERENCES

1. Yang G, Erdman DE, Kodani M, Kools J, Bowen MD, Fields BS, 2011, Comparison of commercial systems for extraction of nucleic acids from DNA/RNA respiratory pathogens. J Virol Methods, 171(1):195-9.
2. Witt S, Neumann J, Zierdt H, Gébel G,Röscheisen C, 2012, Establishing a novel automated magnetic bead-based method for the extraction of DNA from a variety of forensic samples. Forensic Sci Int Genet. 6(5):539- 47.
3. Pan S, Gu B, Wang H, Yan Z, Wang P, Pei H, Xie W, Chen D, Liu G, 2013, Comparison of four DNA extraction methods for detecting Mycobacterium tuberculosis by real-time PCR and its clinical application in pulmonary tuberculosis. J Thorac Dis. 5(3):251-7.
4. Data on file: Coral Clinical Systems.

Symbol keys

25°C / 20°C Store at 20-25°C	Manufacturer	This way up	R1 Lysis/Binding Buffer	R4 Wash Buffer B	Health hazard
Use by (Last day of stated month)	Consult Instructions for use	LOT Batch Number	R2 Magnetic Beads	R5 Elution Buffer	Corrosive
Date of Manufacture	REF Catalogue Number	EC REP Authorised Representative in the European Community	R3 Wash Buffer A	IVD *In vitro* Diagnostic Medical Device	

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Manufactured by:

Coral Clinical Systems
A Division of Tulip Diagnostics (P) Ltd.
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