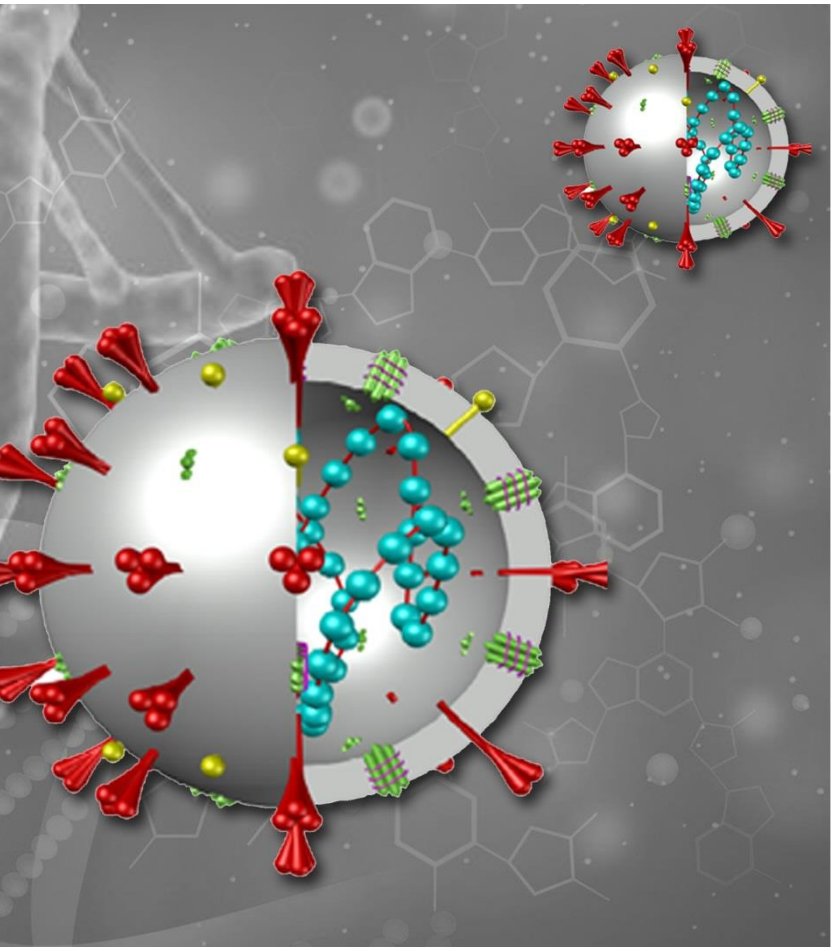

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● Devise ● Develop ● Deliver

AFFIMAG™

COVID-19 VIRAL RNA ISOLATION KIT

BY MAGNETIC BEAD METHOD

AFFIGENIX BIOSOLUTIONS PVT. LTD.

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INTRODUCTION

- Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) causes infection in the lung leading to mild to severe illness and sometimes death.
- Corona virus (2019-nCoV) is an enveloped virus with a positive sense single-stranded RNA genome.
- The genome size of Corona virus (2019-nCoV) is around 31 kilobases, one of the largest among RNA viruses.
- RNA is very unstable and it has to be reverse transcribed to cDNA using reverse transcriptase for early detection of COVID-19 virus in human clinical specimens by polymerase chain reaction technique.
- In order to execute RT-qPCR, an efficient way of capturing and concentrating the RNA from patient samples is the first critical step in the confirmatory tests of the COVID-19 virus.
- The AFFIMAG™ COVID-19 Viral RNA Isolation Kit is designed for the isolation of RNA from samples which includes nasal or throat swabs and sputum for further analysis of nucleic acid amplification and quantitative RT-qPCR study.

PRODUCT DESCRIPTION

- AFFIMAG™ COVID-19 Viral RNA Isolation Kit aids to isolate the RNA from human samples by using either rapid manual or automated instruments.
- The kit components are Affigenix magnetic beads, lysis buffer, wash buffer concentrate, elution buffer, proteinase K, carrier RNA and with or without magnetic separator unit designed to isolate RNA from individuals suspected of COVID-19.
- This kit potentially purifies the total nucleic acid (DNA and RNA) from any biological sample.
- The purified RNA and DNA can be used directly as template for RT-qPCR, PCR, Isothermal PCR or for any other kind of molecular diagnostic reactions.
- Kit is easily adaptable in any 96-well magnetic separator plate or other automated magnetic separation systems.

PERSONAL SAFETY

- Due to highly contagious nature of the corona virus, all works associated with live virus sample should be performed within a BSL-2 biosafety hood with BSL-3 practices.
- Follow the lab biosafety guidelines provided for COVID-19 by CDC.
- All specimens should be treated as potentially infectious and handled with caution.
- Wear proper PPEs (Personal Protective Equipment) such as gloves, head cap, face mask, surgical gowns, eye protection and lab coats while extracting RNA from clinical specimens and also when handling kit reagents, pipettes and equipment.
- Eating, drinking, smoking and contact with skin and eye should be avoided while handling the specimens and reagents.
- All used and unused reagents, plastic wares (reaction tubes/strips) should be packed in biohazard bags, decontaminated and disposed as per the pollution control board norms of the state.

RECOMMENDED WORK PRACTICES

The following recommendations will help to prevent cross contamination.

- Unidirectional workflow in the laboratory layout.
- Use dedicated areas for sample extraction, sample addition and exclusive storage facility.
- Use nuclease free pipette tips with aerosol barriers for liquid handling.

- Separate dedicated equipment (e.g., pipettes, micro centrifuges) and consumables (e.g., micro centrifuge tubes, pipette tips) for assay setup and for handling of extracted nucleic acids.
- Clean and new PPEs are must when setting up the assay.
- All work surfaces must be disinfected thoroughly before and after completion of work.
- Microbial and nuclease contamination of samples and kit components should be prevented.
- Keep reagents and reaction tubes closed as much as possible.

SAFETY PRECAUTIONS AND WARNINGS

- For *in vitro* diagnostic (IVD) use only.
- The kit should be handled by trained technicians.
- Strictly follow the product insert for optimal results.
- Do not use the kit beyond expiry date.

BENEFITS

- Customized RNA isolation kit size - 50 and 100 reactions per kit
- User friendly technique
- Highly efficient nucleic acid isolation

SPECIFICATIONS

- Flexible kit package size to meet all type of laboratory needs

UNIQUE FEATURES

- This kit potentially purifies the total nucleic acid (DNA and RNA) from any biological sample.

  Note: To remove the DNA content from the final elute, treat the final elute with DNase I for 5- 10 mins and inactivate the enzyme at 70°C for RNA isolation.
- Developed in ISO 17025:2017 NABL accredited laboratory

  Note: Supply format may change depending on the end user requirement.

SAMPLE TYPE

Check the CDC website for guidance on specimen collection handling and storage (https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html)

- Respiratory specimens:

  Bronchoalveolar lavage, tracheal aspirate, sputum, nasopharyngeal swab and oropharyngeal swab
- Serum/plasma/blood

SAMPLE HANDLING AND STORAGE

- Samples collected with swabs made of calcium alginate are not acceptable.
- Hemolysed serum samples should be avoided.
- Respiratory and serum samples should be kept at 4°C for no longer than 3 days and for long term storage, store at -70°C.
- Store the extracted RNA at -70°C or lower.
- Follow local and national guidelines for transporting the samples.

EQUIPMENT AND CONSUMABLES

The equipment to be used for COVID-19 RNA isolation should be calibrated and maintained. The equipment should be cleaned and decontaminated prior to use.

The list of equipment and consumables required but are not limited to

Equipment

- Vortex mixer
- Laminar air flow hood
- Heating block or Water bath
- Magnetic separator*
- Biosafety hood (BSL-2)
- PPEs (Personal Protective Equipment's)

Consumables

- Micro centrifuge tubes
- Micropipettes (2, 10, 100, 200 and 1000 µL).
- Racks for micro centrifuge tubes
- Disposable powder free gloves and surgical gowns
- Micropipette tips

Chemicals

- 100 % Ethanol
- DNase 1*

Note : *Provided depending on the laboratory requirement.

AFFIMAG™ COVID-19 VIRAL RNA ISOLATION KIT PROTOCOL

RNA ISOLATION PRINCIPLE

AFFIMAG™ COVID-19 viral RNA isolation kit is devised, designed and developed for the isolation of viral RNA from cell-free body fluids such as bronchoalveolar lavage, tracheal aspirate, sputum, nasopharyngeal swab and oropharyngeal swab washes by using magnetic beads and unique buffer system. The samples are subjected to disruption using lysis buffer containing chaotropic agents to lyse the cell wall and release the components (nucleic acid) from it. The released nucleic acid will then specifically bind to paramagnetic beads which will be captured using magnetic stand. Cycles of wash buffer containing alcohol is used to remove the contaminants. The highly pure nucleic acid is eluted using low salt elution buffer. The obtained highly pure product can be directly used for downstream applications like PCR and RT-qPCR.

ASSAY SETUP REQUIREMENTS

Primary sample	A respiratory sample (sample from nose or throat swab) in viral transport media (VTM)
Storage	All respiratory specimens should be kept at 4°C for no longer than 3 days and for long term storage store at -70°C.
Requisitions	Test requisitions should be accompanied with samples.
Rejection criteria	Low sample volume, contaminated sample, sample not collected in proper container

AFFIMAG™ COVID-19 VIRAL RNA ISOLATION KIT REAGENTS

Component name	Code	Component description	Storage	Volume for 50 Preparations	Volume for 100 Preparations
Lysis buffer	AFF001	Breaks the cell wall	RT	5.5 mL	11 mL
Wash buffer 1 concentrate	AFF002	Remove the contaminants from the solution	RT	11 mL	22 mL
Wash buffer 2 concentrate	AFF003	Remove the contaminants from the solution	RT	9 mL	18 mL
Elution buffer	AFF004	Separate out the desired nucleic acid from the beads	RT	5 mL	10 mL
Carrier RNA*	AFF005	Nucleic acid precipitation	–20°C	1 vial	1 vial
Affi-beads or paramagnetic beads	AFF006	Magnetic beads can adsorb nucleic acids from proteins and other impurities.	RT	1.05 mL	2.1 mL
Proteinase K*	AFF007	Component with Lysis buffer to degrade proteins	–20°C	1 vial	1 vial
Magnetic [a] separator	NA	To capture magnetic particles with the bound nucleic acid.	RT	1 unit	1 unit

[a] Supplied separately only upon request.
*Shipping at room temperature, store at 2-8°C as soon as receipt of the shipment.
*Store at –20°C in aliquots after reconstitution.

REAGENT STORAGE, HANDLING AND STABILITY

All the reagents (except carrier RNA & proteinase K) and components of AFFIMAG™ COVID-19 Viral RNA Isolation Kit should be stored at RT and is stable for 12 months from the date of manufacture.

RNA EXTRACTION AND ISOLATION PROCEDURE

This protocol is designed for isolation of RNA from suspected COVID-19 sample in reaction tubes with suitable magnetic separator.

BUFFER PREPARATION

Wash Buffer-1 (Concentrate): Wash Buffer-1 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle and in the below table. Wash Buffer-1 is stable for 1 year upon appropriate storage at room temperature (15–25°C).

Components	Volume for 50 preparations (mL)	Volume for 100 preparations (mL)
Wash Buffer-1 (Concentrate)	**11**	**22**
Ethanol	**14.5**	**29**
Final Volume	**25.5**	**51**

Wash Buffer-2 (Concentrate): Wash Buffer-2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle and in below table. Wash Buffer-2 is stable till the expiration date on appropriate storage at room temperature (15–25°C).

Components	Volume (mL) for 50 Preparations	Volume (mL) for 100 Preparations
Wash Buffer-2 (Concentrate)	**9**	**18**
Ethanol	**21**	**42**
Final Volume	**30**	**60**

CARRIER RNA PREPARATION

Before first use of the kit, add the following required volume of elution buffer to the carrier RNA vial and mix well. Store the dissolved carrier RNA solution in aliquots at -20°C.

Components	For 50 Preparations	For 100 Preparations
Carrier RNA	1 vial	1 vial
Elution Buffer	420 µL	840 µL

PROTEINASE K PREPARATION

Before use of the kit, add the following required volume of elution buffer to the proteinase K vial and mix well. Store the dissolved proteinase K solution in aliquots at -20°C.

Components	For 50 Preparations	For 100 Preparations
Proteinase K	1 vial	1 vial
Elution Buffer	510 µL	1020 µL

LYSIS BUFFER PREMIX PREPARATION

The premix is stable only for 48 hours at 2-8°C. Use freshly prepared lysis buffer premix during the process.

No. of Preparations	Components				
	Lysis buffer (mL)	Carrier RNA (µL)	Proteinase K (µL)	Affi-beads (µL)	Total volume (mL)
1	0.1	8	10	20	0.138
10	1	80	100	200	1.38
20	2	160	200	400	2.76
30	3	240	300	600	4.14
40	4	320	400	800	5.52
50	5	400	500	1000	6.9
60	6	480	600	1200	8.28
70	7	560	700	1400	9.66
80	8	640	800	1600	11.04
90	9	720	900	1800	12.42
100	10	800	1000	2000	13.8

PROCEDURE

1. Add 200 µL of VTM containing nasal/throat swab sample to a suitable 2mL DNase/RNase free micro centrifuge tube
2. Add 138 µL lysis buffer premix to the sample. Vortex the mixture and incubate for 10 mins at 56°C on thermo mixer.

3. Add 300 µL of ethanol (96 – 100%) into the lysate and vortex the mixture. Incubate the lysate mixture in thermo mixer for 5 mins at 37°C at 900 RPM.
4. Place the centrifuge tube on the magnetic separator. Wait at least 1 min for the magnets to attract the beads.
5. Discard the supernatant from the tube using a pipette without disturbing the magnet bound beads.
6. Remove the micro centrifuge tube from the magnetic separator. Resuspend the beads in 500 µL wash buffer-1 and incubate in thermomixer at 37°C at 900 RPM for 3mins.
7. Settle the beads by placing the tube on a magnetic stand for a minute and discard the supernatant and add 500 µL wash buffer-2 and incubate in thermomixer at 37°C at 900 RPM for 3mins.
8. Place the centrifuge tube on the magnetic separator and wait at least 1 min to discard the supernatant completely.
9. Dry the beads at 56°C for approximately 3 mins or untill it gets dry.

   Note: Remove the wash buffer as much as possible as it contains ethanol and other trace materials.

   Note: Do not leave it for much longer to dry completely as the beads become difficult to resuspend.
10. Add 50 µL elution buffer to magnetic beads and incubate for 4-5 mins with gentle shaking at 56°C.
11. Perform magnetic separation using magnetic separator for at least 2-3 mins and carefully collect the nucleic acid elutes into a new DNase/RNase-free centrifuge tube and store at -80°C or below or use it immediately.

    Note: If the liquid is found on the tube wall or cap during operation, centrifuge the tube briefly so that the liquid can get back to the bottom of the tube.
12. The final elute contains total nucleic acid (DNA and RNA) from the biological sample. To remove the DNA content from the final elute, treat the final elute with DNase I for 5- 10 mins and inactivate the enzyme at 70°C.
13. Appropriate volume of final elute can be directly used for RT-qPCR reaction.

QUALITY CONTROL

In accordance with Affigenix's ISO certified quality systems, each lot of the kit is tested against identified specifications to ensure consistent product quality.

BIOHAZARD SAFETY REFERENCES

www.who.int/csr/resoiurces/publications/biosafty/biosafety7.pdf

www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

REFERENCES

Albertoni GA, Arnoni CP, Araujo PR, et al. Magnetic bead technology for viral RNA extraction from serum in blood bank screening. Braz J Infect Dis 2011; 15(6):547-552.

ORDERING INFORMATION

Catalogue No.	Product Name	Preparations
AFFIMAG - 50	AFFIMAG™ COVID-19 VIRAL RNA ISOLATION KIT BY MAGNETIC BEAD METHOD	50
AFFIMAG - 100	AFFIMAG™ COVID-19 VIRAL RNA ISOLATION KIT BY MAGNETIC BEAD METHOD	100

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