IVD

Nucleic Acid Extraction Reagent

Instruction for Use

For Professional Use Only

Product Name

Nucleic Acid Extraction Reagent

Catalogue Reference Number

2001FC

Model

MNP-100F

Packing Specifications

Specification: 100 Preps/kit

Product Description

The Nucleic Acid Extraction Reagent is designed for rapid and reliable isolation of total nucleic acid from swab, saliva, and other body fluids. The Nucleic Acid Extraction Reagent provides high-quality RNA or DNA, which is suitable for direct use in most downstream applications such as amplifications and enzymatic reactions. This system can be easily adapted to automated systems or centrifugation systems. The procedure can be scaled up or down, allowing purification from various amounts of starting material.

Intended Use

This kit with reagents for 100 isolations was designed for the isolation DNA/RNA from upper respiratory tract specimens (including oral swabs, throat swabs, nasal swabs, nasopharyngeal extracts), lower respiratory tract specimens (including bronchoalveolar lavage fluid, alveolar lavage fluid).

The kit is "For Professional Use Only" by trained and validated laboratory personnel. Read these instructions carefully before use the kit.

Kit Storage and Handling

The Nucleic Acid Extraction Reagent (100 Preps) can be stored at room temperature (10°C to 30°C, which is equivalent to 50°F~86°F) for 12 months from the date of manufacture. Please see label for expiration date.

Specimen Collection and Handling

Typical clinical samples are throat swab and bronchoalveolar lavage.

Throat Swab: Use the plastic rod swab with polypropylene fiber head to wipe the bilateral pharyngeal tonsils and the posterior pharyngeal wall at the same time, immerse the swab head into the tube containing physiological saline, discard the tail, and tighten the tube cover.

Bronchoalveolar Lavage: Collect bronchoalveolar lavage for test. The collected sample should be used for detection as soon as possible. If the sample need to be transferred cannot be detected immediately, please store it at low temperature.

The sample can be stored for 24 hours at 2°C to 8°C and for a long time below -70°C. It can also be stored in refrigerator at -20°C temporarily.

Samples shall be transported at low temperature in accordance with biosafety regulations.

Principle of the Procedure

The isolation procedure is based on magnetic beads technology and can be divided into the following steps:

1. Lysis and stabilization of the sample with lysis-binding buffer.
2. Magnetic beads are added to specimens lysate, and total nucleic acids (RNA, DNA) are bound onto the magnetic beads during incubation.
3. Magnetic beads are separated by centrifugation or magnetic separator and unbound material is removed by washing.
4. Nucleic acids (RNA and DNA) are eluted from the magnetic beads. At this stage, the nucleic acids can be used for DNA and RNA analysis.

Kit Contents and Preparation of Working Solution

Name of Component	Specification	No. of bottle	Working Solution	Storage Condition
MNP Lysis-binding Buffer	54mL/bottle	1 bottle	Add 20mL of isopropanol before use	Store at 10°C to 30°C (50°F to 86°F)
MNP Magnetic Beads	9mL/bottle	1 bottle	Ready-to-use	
MNP Washing Buffer I	32mL/bottle	1 bottle	Add 59mL ethanol before use	
MNP Elution Buffer	22mL/bottle	1 bottle	Ready-to-use	

Note: The components in different batches of reagents can be used interchangeably within the validity period.

Materials and Equipment to be Supplied by User:

Reagents

Reagents	Volume
Isopropanol	20mL
Ethanol (96%~100%)	59mL
Washing buffer II (75% ethanol)	80mL

Equipment

- Magnetic separator.
- Vortex mixer
- Roller incubator or other suitable instrument; incubation temperature: 15℃ to 25 ℃
- Automatic extraction system (optional)

Before first use: prepare working solutions

- Add 20mL of isopropanol to MNP Lysis-binding Buffer for a working solution.
- Add 59mL ethanol (96%~100%) to MNP Washing Buffer I for a working solution.
- Prepare 75% ethanol as MNP Washing Buffer II.

Procedure

Pre-treatment of samples: If samples are deep-frozen, thaw samples carefully before use.
Sample volumes：
In the following, the detailed procedures for DNA/RNA isolation from 600µL of MNP Lysis-binding Buffer corresponding to 300µL of original sample material are described. If sample volumes more or less than 300µL are to be processed, the procedure described can be linearly downscaled.

Manual Extraction Protocol

Step	Action
1	To prepare a suitable tube [e.g.1.5 mL or 2 mL Eppendorf tube], add 600uL MNP Lysis-binding Buffer working solution, and 75μL beads, 300μL sample (if samples were deeply frozen, it need to be prewarmed to 15°C to 25°C before use). ● Note: The MNP Magnetic Beads should be thoroughly mixed (e.g., by vortexing) before use. When dispensing MNP Magnetic Beads to tubes, mix briefly every 20 seconds to prevent the sedimentation of MNP Magnetic Beads which may affects the extraction results.
2	Lysis and bind nucleic acids to MNP Magnetic Beads by incubation for 15 min at room temperature on a roller incubator.
3	Separate MNP Magnetic Beads by using a magnetic separator, and discard the supernatant.
4	Wash MNP Magnetic Beads with MNP Washing Buffer I by using a magnetic separator and a suitable tube[e.g. 1.5mL or 2 mL Eppendorf tube]. ● Washing steps are carried out as follows: suspend separated MNP Magnetic Beads by pipetting thoroughly in 700μL MNP Washing Buffer I.
5	Wash MNP Magnetic Beads with MNP Washing Buffer II (75% ethanol) by using a magnetic separator and a suitable tube[e.g. 1.5 mL or 2 mL Eppendorf tube]. ● Washing steps are carried out as follows: suspend separated MNP Magnetic Beads by pipetting thoroughly in 700μL MNP Washing Buffer II (75% ethanol).
6	Separate MNP Magnetic Beads by using a magnetic separator.
7	Remove supernatant completely, let the cap tube open and the remaining MNP Washing Buffer II evaporate.
8	Resuspend MNP Magnetic Beads in 100μL MNP Elution Buffer by incubation for 5 min at room temperature.
9	Separate MNP Magnetic Beads using a magnetic separator and transfer the supernatant to a fresh tube. Repeat separation with the supernatant to remove residual magnetic particles completely and transfer supernatant into a fresh tube.
10	Further process isolated DNA/RNA, or store at -20°C to -80°C.

Automatic extraction by using machine (magnetic rods type, *eg.*, KingFisher™ Flex Magnetic Particle processor)

Note: The reagents dispense in the deep well plate may differ due to the different machine, following instructions is only Principle of the Procedure.

1. Prepare a 96-well deep well plate (2.2 mL).
2. Dispense reagents as shown in the table below:

Working solutions	Volume
MNP Lysis-binding Buffer	600μL/well
MNP Washing Buffer I	700μL/ well

MNP Washing Buffer II (75% ethanol)	700μL/ well
MNP Elution Buffer	100μL/ well
MNP Magnetic Beads	75μL/ well

3. Mix the samples thoroughly, and then add 300 μL of the sample to the MNP Lysis-binding Buffer. (if samples were deeply frozen, it need to be prewarmed to room temperature before use).
4. Automatic extraction with the following settings:

Steps	Task	Air-dry time (min)	Mixing time (min)	Magnetic suction time (min)	Mixing speed	Temperature (℃)
1	Transfer beads	0	1	1	high	RT
2	Lysis and binding	0	15	1	high	RT
3	Washing I	0	1	1	high	RT
4	Washing II	0	1	1	high	RT
5	Elution	0	5	1	high	RT
6	Discard beads	0	1	0	high	RT

Note: RT：Room temperature

5. After the procedure is over, the DNA / RNA in the MNP Elution Buffer is ready-to-use.

Limitations of the Procedure

This product is only suitable for upper respiratory tract specimens (including oral swabs, throat swabs, nasal swabs and nasopharyngeal extracts) and lower respiratory tract specimens (including bronchoalveolar lavage fluid and alveolar lavage fluid); performance for other types of samples or samples in other preservation buffers cannot be guaranteed.

Performance Characteristics

1. Yields of viral RNA isolated from biological samples are normally less than 1 μg and therefore difficult to determine photometrically. Keep in mind that this kit extracts total nucleic acid from sample, including DNA and RNA, thus quantitative RT-PCR is recommended for determination of viral RNA yield.

2. Repeat the extraction 10 times for the reference sample, and then the purified RNA or DNA can be determined using quantitative RT-PCR detection kit. Variation coefficient (CV) of Ct values should be less than 10%.

Warnings and Precautions

1. Before use, carefully check whether the reagent components are complete.

2. Frozen samples should be thawed and mixed before use.

3. The detected sample shall be deemed as having infectious substances, and operation and treatment shall both conform to the requirements of relevant laws and regulations.

4. Sample treatment in the biosafety cabinet, wear work clothes and disposable gloves during the test process and use the dump tubular pipettor. The pipettes used in the experiment should be directly put into the waste tank containing the disinfectant, and discarded after being sterilized together with other waste.

5. It is recommended to perform UV disinfection of the nucleic acid extraction instrument for 20 minutes before and after the experiment.

6. A small amount of magnetic beads may remain during elution. Avoid sucking magnetic beads when sucking DNA / RNA for subsequent operations.

7. This kit contain guanidine salts (e.g., guanidine thiocyanate and guanidine hydrochloride) that may produce hazardous gases when combined with bleach (sodium hypochlorite) and/or strong acids.

8. After the completion of experiment, it shall use 10% hypochlorous acid, 75% alcohol or ultraviolet radiator for disinfection.

9. The operators should have operational experience and have received professional training.

10. This kit is only used for in vitro diagnosis.

References

1. Brestovac B, Wong ME, Costantino PS, etal. A rapid DNA extraction method suitable for human papillomavirus detection. J Medical Virol. 2014, 86(4): 653-7.
2. Berensmeier S. Magnetic particles for the separation and purification of nucleic acids. Appl Microbiol Biotechnol. 2006, 73(3): 495-504.
3. Katevatis C, Fan A, Klapperich CM. Low concentration DNA extraction and recovery using a silica solid phase. PloS One. 2017, 12(5): e0176848.
4. He H, Li R, Chen Y, etal. Integrated DNA and RNA extraction using magnetic beads from viral pathogens causing acute respiratory infections. Sci Rep. 2017, 7: 45199.

Explanation of Symbols

Symbol	Description
	Catalog number
	Contains sufficient for <n> tests
	Batch code
	Date of manufacture
	Use-by-date
	Manufacturer
	In vitro diagnostic
	Consult instructions for use
	Temperature limit

Manufacturer Basic Information

Manufactured for:
Fosun Pharma USA Inc.
104 Carnegie Center, Suite 204
Princeton, NJ 08540
Tel: (866) 611-3762

Manufactured by:
Yaneng BIOscience (Shenzhen) Co., Ltd.
Room 301, 302, 304, 401A1
Building No.1 Bio-Pharmacy Business Accelerator
14 Jinhui Road, Kengzi Street
Pingshan District, Shenzhen, Guangdong, China

Made in China

Rev 08/2021

The Nucleic Acid Extraction Reagent *Instruction for Use* can be downloaded from the following link: **www.fosunpharmausa.com/covid19/pcr/**