Promotor®

Viral Nucleic Acid Isolation Kit
Package Insert

REF P121-1051	English

For extracting RNA from various types of specimens using spin column method.

For professional in vitro diagnostic use only.

INTENDED USE

Viral Nucleic Acid Isolation Kit is designed for rapid isolation and purification of nucleic acids from serum, plasma or swabs. The extracted nucleic acids can be used for routine molecular biological operations or clinical molecular diagnosis, such as nucleic acid detection, cloning, sequencing analysis, PCR amplification, molecular hybridization, etc.

PRINCIPLE

With the spin column method, nucleic acids are quickly and efficiently isolated and purified. Nucleic acids bind to spin column in the conditions of high salt and low pH Buffer L, then were eluted in low salt and high pH Buffer TE. Buffer L and ethanol create appropriate conditions for binding of nucleic acids to the Spin Columns. Contaminations (potential PCR inhibitors) like salts, metabolites, and soluble macromolecular cellular components are removed in simple wash steps with alcoholic buffers Buffer WA and Buffer WB. The nucleic acids are eluted in Buffer TE and are ready-to-use in subsequent reactions.

PRECAUTIONS

Handling Requirements

- For professional in vitro diagnostic use only. Do not use after the expiration date.
- Use dedicated equipment and supplies, pipettes and pipette tips.
- Avoid cross contamination of specimens and reagents for accurate extraction results. Pipette tips should be changed between all liquid transfers.
- Always use aerosol barrier pipette tips. The pipette tips must fit the barrel properly to avoid leakage, inaccurate measurements or looseness while the pipettes are in use.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection as needed while handling all specimens, kit reagents, and mixes.
- Change gloves regularly throughout the procedure.
- Clean the entire working area (working table surfaces and all instruments) with diluted bleach equivalent to 1% sodium hypochlorite, followed by purified water and 70% ethanol solution.
- Routinely monitor to prevent contamination of the laboratory and equipment.
- Do not mix reagents with other kits of different lot numbers.
- Do not use reagents from different lots in the same test.
- Wash hands thoroughly when finished handling specimens and reagents.
- Strictly follow the instructions in this package insert.
- Keep reagents away from skin, eyes and mucous membranes. If contact occurs, wash the affected area with large amounts of water immediately. If the reagents spills, dilute the spilled reagents with water before wiping them up.
- All equipment should be calibrated and maintained regularly according to the instructions.

Laboratory Procedures

- Please comply with the local regulations about medical laboratories.
- Handle all contaminated materials, specimens, and reagents following established good laboratory working practices. All specimens should be supposed to be potentially infectious and handled properly. Since the sensitivity and titer of potential pathogens in the specimen material varies, the operator must optimize pathogen inactivation by using lysis solution or taking appropriate measures according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory working area where the kits are handled.
- Do not pipette specimens or reagents by mouth.

Waste Handling

- Dispose unused reagents, contaminated materials, specimens, and waste in accordance with local, state, and national regulations.

STORAGE AND STABILITY

Isolation Kit 1

Unopened kit 1 should be stored at 2-30°C upon receipt. All unopened reagents are stable until the expiration date printed on the box if stored at 2-30°C. Reagents are stable for 3 months after the first opening. Unused reagents should be stored at 2-30°C.

Isolation Kit 2

Unopened kit 2 should be stored at -20±5°C upon receipt. All unopened reagents are stable until the expiration date printed on the box if stored at -20±5°C. The kit 2 reagents are stable for 3 months after the first opening. Unused reagents should be stored at -20±5°C.

SPECIMEN COLLECTION AND PREPARATION

Serum or Plasma

- Collect 5 mL venipuncture whole blood using a disposable syringe.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis.

  Note: Blood should be collected in the tubes using EDTA or ACD as coagulant. Heparinized specimen is not recommended as heparin may inhibit PCR.
- Centrifuge the tube 20 minutes at 2,000-4,000 rpm.
- Transfer serum or plasma for testing.

Cotton Swab

- Collect specimen using aseptic cotton swab and put the swab in a sterilized plastic screw top tube.
- Add 1 mL preservative fluid (virus preservative fluid, isotonic saline solution, tissue culture medium, PBS etc) to the tube with cotton swab containing specimen and shake it gently.

Note: **Specimen Preparation and Transportation**

- **Preparation:** Testing should be performed immediately after specimen collection. Keep specimens at room temperature prior to extraction. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- **Transportation**: Samples shall be transported at low temperature in accordance with biosafety regulations. If specimens are to be shipped, they should be packaged and labeled in compliance with local or international regulations covering the transport of specimens and etiologic agents.

REAGENTS AND COMPONENTS

Materials Provided

Kit contents		Composition	32 tests
kit 1	Buffer L	Guanidine salt,Triton-100	1 × 8 mL
	Buffer WA	Guanidine salt	1 × 9 mL
	Buffer WB	H_2O	1 × 6 mL
	Buffer TE	Tris, EDTA	1 × 1.7 mL
	Collection Tubes (2 mL)	/	32 pcs
	Spin Columns	/	32 pcs
kit 2	Carrier RNA	Carrier RNA	1 ×120 μL

***Note:** For Buffer WA, add 12 mL anhydrous ethanol before use. For Buffer WB, add 18 mL anhydrous ethanol before use.

Materials Required But Not Provided

- Anhydrous Ethanol

- 1.5 mL centrifuge tubes

  Note: Only DNase-free & RNase-free tubes can be used
- Pipette tips

  Note: Only DNase-free & RNase-free pipette tips with a filter element can be used
- Disposable gloves and other protective gear
- High speed centrifuge (can be equipped with centrifugal 1.5 mL centrifugal tube and 2 mL centrifugal tube rotor)
- Vortex Oscillator
- Water bath or Dry bath

DIRECTIONS FOR USE

1. Preparation Procedure:

1) If a precipitate has formed in Buffer L, incubate the buffer at 70°C by using Water bath or Dry bath until the precipitation dissolved.

2) Add 12 mL Anhydrous Ethanol to Buffer WA and add 18 mL Anhydrous Ethanol to Buffer WB, mark the bottles to indicate that the Anhydrous ethanol is added.

3) Incubate the Buffer TE at 56°C for later use.

4) Mix Carrier RNA gently and centrifuge immediately. Then add 80 µL Carrier RNA to Buffer L that has no precipitation, and mix well.

2. Operation Procedure

1) Add 200 µL Buffer L containing Carrier RNA and 200 µL sample to the 1.5 mL centrifugal tube in sequence. Mix immediately for several seconds and incubate at room temperature for 10 minutes.

Note: To avoid cross-contamination, centrifuge the Buffer L for several seconds at low speed before open.

2) Add 200 µL Anhydrous Ethanol to the centrifugal tube, gently invert the centrifugal tube for 3-5 times to mix well.

Note: To avoid cross-contamination between samples, centrifuge the tube several seconds at low speed.

3) Insert the Spin Column into the 2 mL Collection Tube, the mixture that collected in step 2 was added to the Spin Column. Centrifuge 30 seconds at 12000 rpm.

Note: Avoid to contaminate the edge of the Spin column orifice.

4) Discard the filtrate and remove the residual filtrate on the edge of the Collection Tube by using tissues. Insert the Spin Column back into the 2 mL Collection Tube. Add 600 µL Buffer WA to the Spin Column, centrifuge 30 seconds at 12000 rpm.

Note:

1. The filtrate does not need to be completely discarded. The purpose of removing the residual filtrate is to avoid the contamination of the centrifuge.
2. Confirm that Anhydrous Ethanol has been added to Buffer WA before first.

5) Discard the filtrate and remove the residual filtrate on the edge of the Collection Tube by using tissues. Insert the Spin Column back into the 2 mL Collection Tube. Add 600 µL Buffer WB to the Spin Column, centrifuge 1 minute at 14000 rpm.

Note:

1. Confirm that Anhydrous Ethanol has been added to Buffer WB.
2. If the speed of the centrifuge is less than 14000 rpm, used the maximum centrifugal speed to centrifuge 2 minutes.

6) Discard the filtrate and remove the residual filtrate on the edge of the Collection Tube by using tissues. Insert the Spin Column back into the 2 mL Collection Tube. Centrifuge 1 minute at 14000 rpm.

Note:

1. If the speed of the centrifuge is less than 14000 rpm, used the maximum centrifugal speed to centrifuge 2 minutes.
2. Do not ignore this step, otherwise, the purified nucleic acid may be mixed with ethanol that will cause false results.

7) Discard the 2 mL Collection Tube, insert the Spin Column into a clean 1.5 mL centrifugal tube. Add 50 µL Buffer TE (preheated to 56°C) to the central membrane of the Spin Column. Incubate at room temperature for 1 minute, then centrifuge 30 seconds at 12000 rpm.

Note:

1. Only DNase-free and RNase-free 1.5 mL centrifugal tube can be used.
2. Make sure to close the lid of centrifuge or cut off the cover of the tubes to avoid the damage of the centrifuge caused by the falling cover.

8) Discard Spin Column, collect purified nucleic acid for subsequent test or stored at -20±5°C.

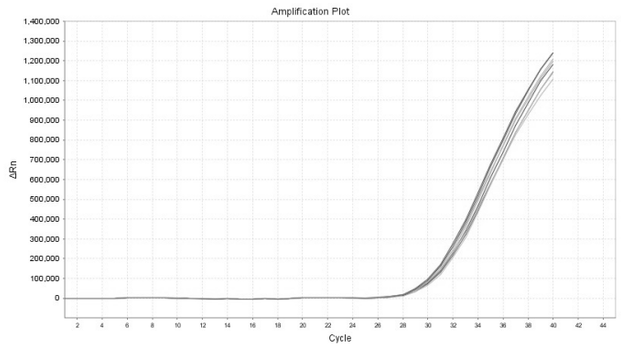
LIMITATIONS

Improper specimen collection, shipping, storage and processing can lead to low concentration and low-purity nucleic acid.

PERFORMACE CHARACTERISTICS

Precision

Precision was evaluated by testing low concentration HCV samples. The samples were extracted by Viral Nucleic Acid Isolation Kit and the eluted RNA was amplified and analyzed on *Applied Biosystems*® 7500 Real-Time PCR System. Ten replicates were performed with the same procedure. All 10 replicates showed positive results, and the CV value was ≤5%.



REFERENCE

Real time quantitative PCR. Genome Res. 6:986–994.

Index of Symbols

	Consult instructions for use	Collection Tubes (2 mL)	Collection Tubes (2 mL)
IVD	*In vitro* diagnostic medical device	Spin Columns	Spin Columns
Σ	Contains sufficient for <*n*> tests	EC REP	Authorized representative in the European Community
Buffer L	Buffer L	LOT	Batch code
Buffer WA	Buffer WA	CE	CE mark
Buffer WB	Buffer WB	REF	Catalogue number
Buffer TE	Buffer TE		Temperature limit
Carrier RNA	Carrier RNA		Use-by date
	Manufacturer		Do not reuse
Insert	Package insert		

***ACON* Biotech (Hangzhou) Co., Ltd.**
No.210 Zhenzhong Road, West Lake District, Hangzhou, P.R.China, 310030

Number: 1151206601
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