Bionew™ Viral RNA Purification Kit

Size: 100 preps **#Cat: BN100-032a**

I. Description

Viral RNA isolation is the most technically demanding and laborious procedure performed in viral research laboratory. The purification procedure usually uses hazardous organic chemicals, which need multiple centrifugations. This will result in significant loss of viral RNA, may badly affect the detection of viral RNA and introduce amplification inhibitors.

Using spin column to purify viral RNA from samples is conventional method now, which required viral RNA was adsorbed to the membrane by centrifuge or vacuum, which bring not only RNA but also cell debris and other precipitate to the membrane. The membrane can be clogged because of the precipitate, then result in inefficient recovery of samples.

A recent developed viral RNA extraction method can be directly used for purification of viral RNA in an “absolutely liquid state”. It is the use of magnetic beads covered with nucleic acid–binding matrices. Its theory is viral DNA/RNA in the solution is specifically adsorbed to the surface of magnetic beads because the following action: (i) shielded intermolecular electrostatic forces, (ii) dehydration of the DNA/RNA and beads surfaces, and (iii) intermolecular hydrogen bond formation in the DNA/RNA–beads contact layer.

This method can farthest eliminate the inhibition in the viral DNA/RNA extraction course, because in an “absolutely liquid state”, viral DNA/RNA is specifically, freely and easily adsorbed to the surfaces of magnetic beads, and the precipitate in the solution can’t make any effect for the absorbance. The absorbance is so strong to experience several times of wash steps to eliminate the impurities from the surface of magnetic beads. Finally the pure viral DNA/RNA is eluted and can be used in PCR or other test directly.

Bionew™ viral RNA purification kit is RNA extraction. It can be widely used in viral RNA purification with samples from CDC, Hospital, Inspection and Quarantine institution, Research Institute, etc. The procedures of viral RNA extraction system containing: sample lysis and viral RNA releasing, viral RNA binding to surface of magnetic beads, 2 times washing to eliminate the impurity from the surface of magnetic beads, eluting viral RNA from the surface of magnetic beads.

For more information, see the www.bionewtech.cn or contact Bionewtech Technical Service: service@bionewtech.cn .

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II. Components and Storage

Components	Size（100 preps）
Magnetic Beads Solution	0.88 ml×1 bottle
Proteinase K Solution	1.4 ml×1 tubes
Carrier RNA Solution	280μl×1 tube
Lysis Buffer	11ml×1 bottle
Wash 1 Buffer	11 ml×1 bottle
Wash 2 Buffer	11 ml×1 bottle
Elution Buffer	1.4ml×4 tubes
2ml RNAse-Free Tubes	200

Upon receipt, store Carrier RNA Solution at -20℃, store Lysis Buffer at 15-25℃, and store all other reagents at 4℃.

III. Applicability

The kit can be directly used for Viral RNA purification from samples: **serum**, **plasma**, **swab solution, cell-culture supernatants**, **supernatants of tissue solution, etc.**

Ⅳ. Materials Needed

a) Reagents: 100% Isopropyl Alcohol

b) RNAse-Free Expendables: 1.5ml Centrifuge Tube, Tips and Filtered Tips of 10μl, 200μl, 1000μl. To make the RNAse-free expendable, customer can process the expendable with 0.1% DEPC over night, then process the DEPC treated expendable in autoclave for 20min,121℃, 1.01E+5 Pa.

c) Customer can also purchase RNAse-free expendables directly from **Bionewtech.**

d) Instruments: Pipettor(10μl,200μl,1000μl); Vortex Agitator, Constant Temperature Equipment

Ⅴ. Protocol

(1) Preparation Protocol before use

1.1 For the [Proteinase K Solution], make aliquots of 250μl in the RNAse-Free 1.5 ml tubes (the tubes were prepared by customer), and store at 4℃. Do not freeze-thaw the aliquots of [Proteinase K] more than 3 times.

1.2 For the tube of [Carrier RNA Solution], make aliquots of 50μl in the RNAse-Free 1.5 ml tubes (the tubes were prepared by customer), and store at **-20℃**. Do not freeze-thaw the aliquots of [Carrier RNA] more than 3 times.

1.3 For the bottle of 11ml [Wash 1 Buffer], add 11ml Isopropyl alcohol to the bottle and mix it by the bottle up and down, mark it to confirm the addition of isopropyl alcohol. It is the final [Wash 1 Buffer]. Screw the cap closely and store at 4℃ for up to expired date.

1.4 For the bottle of 11ml [Wash 2 Buffer], add 11ml Isopropyl alcohol to the bottle and mix it by the bottle up and down, mark it to confirm the addition of isopropyl alcohol. It is the final [Wash 2 Buffer]. Screw the cap closely and store at 4℃ for up to expired date.

1.5 For the bottle of 0.88ml [Magnetic Beads Solution], add 4.7ml Isopropyl alcohol to the bottle and mix it by 1min vortex, mark it to confirm the addition of isopropyl alcohol. It is the final [Magnetic Beads Solution]. Screw the cap closely and store at 4℃ for up to expired date.

(2) Purification Protocol

2.1 At cold room temperature, if the [Lysis buffer] forms a precipitate, warm the solution in 37℃ for 20mins to solve the precipitate completely. For N samples, use 2ml N RNAse-free tubes (for short "tubes") and separately marked them.

2.2 Take N×100μl [Lysis buffer], N×2.5μl step1.2 [Carrier RNA], and mix it.

2.3 Add 12.5μl step1.1 [Proteinase K] into every tube,

2.4 Add 50μl sample into corresponding tube using 200μl filtered tip.

2.5 Add 100ul step2.2 mixed [Lysis buffer],

2.6 Add 50μl [Magnetic Beads Buffer] into every tube, vortex for 20s, incubate at room temperature for 8 minutes.

2.7 Place the N tubes on magnetic stand for 3 minutes to adsorb the magnetic beads to the wall of tube because of magnetic force, and then discard supernatant in every tube.

2.8 Remove the tubes from magnetic stand; add 200μl step1.3 [Wash 1 Buffer] into every tube, vortex 10s.

2.9 Place the tubes on magnetic stand for 2 minutes, and then discard supernatant in every tube.

2.10 Remove the tubes from magnetic stand; add 200μl step1.4 [Wash 2 Buffer] into every tube, vortex 10s.

2.11 Place the tubes on magnetic stand for 2 minutes, and then discard supernatant in every tube.

2.12 Open the lids to dry every tube at room temperature for 3minutes, and then remove again the residual solution at bottom of every tube.

2.13 Add [Elution Buffer] 50μl, mix magnetic beads in every tube by slowly drawing and pumping with 200μl filtered tip, and then incubate every tube at 56℃ for 3 minutes.

2.14 Place the tubes on the magnetic stand for 2 minutes,

2.15 Transfer every eluate to a new 1.5ml RNAse-Free tube (prepared by customer). It is the eluted Viral RNA for every sample; it can be used for next detection at once, or stored at -20℃ for 1 week or stored at -70℃ for long time.

Ⅵ. Troubleshooting

For questions not addressed here, please visit Bionewtech web site: www.bionewtech.cn or

contact Bionewtech Technical Service: service@bionewtech.cn .

Symptoms	Possible Causes	Comments
Little or no RNA in the eluate	Forgot adding Carrier RNA in the purification protocol;	Repeat the purification with new samples and confirm to add Carrier RNA;
	Carrier RNA degraded	Change new Carrier RNA, store Carrier RNA at -20℃ and do not freeze-thaw Carrier RNA more than 3 times.
	RNA degraded	RNA is often degraded by RNAse in the starting material. Ensure samples are process timely and effectively after collection
	RNAse contamination in the [Elution Buffer]	Change one new tube of [Elution Buffer]
	Sample frozen and thawed more than 1 time	Always used fresh samples or samples thawed only 1 time
	Low concentration of virus in the sample	Concentrate the sample volume to 100μl using a micro centrifuge.
Beads in final elution	Beads is occasionally transferred by rapid pipetting	Vortex or mix solution, place in the magnetic stand and transfer eluate to new tube again.
Inconsistent Ct of duplicated samples	Inconsistent amounts of magnetic beads	Be sure to vortex the magnetic beads solution vigorously before use and vortex beads in the binding step vigorously.
	Inconsistent sample is used	Be sure to mix the sample equably before use.

Ⅶ. Composition of Buffers and Solutions

Elution Buffer: DEPC H2O

Ⅷ. Related Products

Bionew™ Whole Blood DNA Purification Kit	**#Cat: BN100-011a**
Bionew™ Tissue DNA Purification Kit	**#Cat: BN100-012a**
Bionew™ DNA Micro Purification Kit	**#Cat: BN100-014a**
Bionew™ Plasmid DNA Purification Kit (Medium Quantity)	**#Cat: BN100-015a**
Bionew™ Bacteria DNA Purification Kit	**#Cat: BN100-016a**
Bionew™ Viral DNA Purification Kit	**#Cat: BN100-031a**
Bionew™ Viral RNA Purification Kit	**#Cat: BN100-032a**
Bionew™ Viral DNA/RNA Purification Kit	**#Cat: BN100-033a**
Bionew™ Cells Total RNA Purification Kit	**#Cat: BN100-021a**
Bionew™ Tissue Total RNA Purification Kit	**#Cat: BN100-022a**
Bionew™ Whole Blood Total RNA Purification Kit	**#Cat: BN100-023a**
Bionew™ Lockable Magnetic Stand	**#Cat: BN200-011a**