Bionew™ Tissue Total RNA Purification Kit

Size: 100 preps **#Cat: BN100-022a**

I. Description

Nucleic acid isolation is the most technically demanding and laborious procedure performed in molecular cloning laboratory. At first purification procedure usually used phenol、chloroform, etc. These hazardous organic chemicals, need multiple centrifugations and will result in significant loss of nucleic acid, may affect the enzyme reaction, reverse transcription and PCR.

Using spin column to purify total RNA from samples is conventional method now, which required 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. Especially for tissue sample, after grinding tissue sample and adding some lysis buffer to make a tissue lysate, the lysate is always containing some precipitate.

A recent developed RNA extraction method can directly use culture cells as the samples. It is the use of magnetic beads covered with nucleic acid–binding matrices. Its theory is nucleic acid (DNA and 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 RNA extraction course, because nucleic acid in the lysate is specifically adsorbed to the surfaces of magnetic beads but the precipitate in the lysate isn't adsorbed. Then after 2 times of wash steps for the magnetic beads the other impurities are eliminated, finally the pure RNA is eluted and can be used in RT-PCR directly.

Bionew™ cells total RNA purification kit is the excellent kit which uses magnetic beads for RNA extraction. It can be widely used in RNA purification with sample from Research Institutions, University, Life Science and Industrial Markets. The procedures of total RNA extraction system contains: lysis and RNA release, RNA binding to surface of magnetic beads, 2 times of wash and RNA elution.

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	1.1ml×2 tubes
Lysis Buffer	11 ml×1 bottle
Wash 1 Buffer	22 ml×1 bottle
Wash 2 Buffer	22 ml×1 bottle
Elution Buffer	11 ml×1 bottle
Proteinase K Solution	1.4ml×2 tubes
Enhancer 1	550μl×1 tube
Enhancer 2	550μl×1 tube
Activator	550μl×1 tube

Upon receipt, store Proteinase K Solution, Enhancer 1, Enhancer 2 and Activator at -20℃, store all other reagents at room temperature until Expiry Date.

III. Applicability

The kit can be directly used for RNA purification from the culture cells. For culture cells, centrifuge with 800g, 10min to concentrate cells is necessary.

Ⅳ. Materials Needed

a) Reagents: 100%Alcohol，100% Isopropyl Alcohol

b) RNAse-Free Expendables: Centrifuge Tube of 1.5ml, 2ml, 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 (recommended metal bath)

Ⅴ. Tissue Pretreatment

a) Weight 10mg tissue, adding liquid nitrogen to freeze tissue, and grind tissue to powder;

b) After volatilization of liquid nitrogen, transfer tissue powder to 200ul [Lysis Buffer], vortex 1 min, have a pretreatment at room temperature for 20min;

c) Centrifuge with 4000g, 2min, then transfer supernatant for next RNA purification;

d) Pre-treat every tissue sample as above.

Ⅵ. Protocol

(1) **Preparation Protocol**

1.1 For the bottle of 22ml [Wash 1 Buffer], add 11ml Alcohol, 11ml Isopropyl alcohol to the bottle and mix it by the bottle up and down, mark it to confirm the addition of alcohol and isopropyl alcohol. It is the final [Wash 1 Buffer]. Screw the cap closely and store at 2~8℃ for up to 6 months.

1.2 For the bottle of 22ml [Wash 2 Buffer], add 22ml Isopropyl alcohol to the bottle and mix it by the bottle up and down, mark it to confirm the addition of alcohol and isopropyl alcohol. It is the final [Wash 2 Buffer]. Screw the cap closely and store at 2~8℃ for up to 6 months.

1.3 Before use vortex [Magnetic Beads Solution] for 15s to mix equably. The dosage of beads is 20μl beads/sample, so for N samples take N×20μl beads into new centrifuge tube, then take

N×200μl isopropyl alcohol into this tube, vortex the solution 15s to mix equably. It is the final [Magnetic Beads Solution].

1.4 If the [Lysis Buffer] forms a precipitate, warm the solution in 56℃ for 10mins to solve the precipitate completely.

1.5 For frozen samples and reagents take out to room temperature to unfreeze, then mix equably.

1.6 Before use for N samples, take N×40μl [Elution Buffer], mix equably with N×5μl [Enhancer 1], N×5μl [Enhancer 2]. It is the final [Elution Buffer].

(2) **Purification Protocol**

2.1. For N samples, use N RNAse-free centrifuge tubes (2ml) and separately marked it; add 25μl [Proteinase K Solution].

2.2. Add 100μl sample using different 200μl tips

2.3. Add 100μl [Lysis Buffer] using 200μl tips, vortex for 5s, incubate the sample at 56℃ for 15 minutes.

2.4. Add 220μl step1.3 [Magnetic Beads Solution] using 200μl tips, vortex for 10s to mix it equably, incubate the sample at 56℃ for 3 minutes.

2.5. Place the N centrifuge tubes(2ml) containing sample mixture to the magnetic stand for 3 minutes, the magnetic beads are adsorbed to the wall of tube because of magnetic force, then discard all supernatant using different 1000μl tips.

2.6. Remove the tubes from the magnetic stand, add 400μl step1.1 [Wash 1 Buffer] using 1000μl tips, vortex 5s.

2.7. Place the tubes to the magnetic stand for 2 minutes, and discard all supernatant using 1000μl tips (permission of using the same 1000μl tip).

2.8. Remove the residual solution using 200μl tips.

2.9. Remove the tubes from the magnetic stand; add 400μl step1.2 [Wash 2 Buffer] into every tube with different 1000μl tips, mixed every sample by slowly drawing and pumping by 1000μl pipettor. Note: Don't let tips touch to residual solution on the upside of tube.

2.10. Use N new RNAse-free centrifuge tubes(2ml), marked as step 2.1, transfer every mixture of step 2.9 into every new marked 2ml centrifuge tube

2.11. Place the tubes to the magnetic stand for 2 minutes, and discard all supernatant using 1000μl tips (permission of using the same 1000μl tip).

2.12. Open the lids of tubes and dry at room temperature for 3minutes, and then remove again the residual solution at the bottom of tubes with 200μl tips

2.13. Add 50μl step1.6 [Elution Buffer], mix every sample by slowly drawing and pumping by 200μl pipettor, incubate at 37℃ for 30 minutes.

2.14. Add 5μl Activator into every sample; incubate at 65℃ for 10 minutes.

2.15. Place the tubes to the magnetic stand for 2 minutes, Transfer the supernatant to the new RNAse-free centrifuge tubes (1.5ml). It is the eluted total RNA, it can be used for next detection at once or stored at -20℃ for 1 week or or stored at -70℃ for long time.

2.16.The yield and purity can be determined by spectrophotometer. For RNA:

RNA Concentration = （OD_{260}-OD_{320}）× 40 μg/ml

RNA Purity = （OD_{260}-OD_{320}）/（OD_{280}-OD_{320}），

When using the frozen samples, its concentration and purity is decreased comparing with fresh samples, so the range of purity of pure dsDNA is between 1.7 and 2.0.

Ⅶ. Troubleshooting

For questions not addressed here, please contact Bionewtech. Web site: www.bionewtech.cn

Email: service@bionewtech.cn

Symptoms	Possible Causes	Comments
Poor yield	Too little sample used，or the initial amount of RNA in the sample is poor.	Increase initial amount of sample.
	The sample is frozen for a long time, and the RNA has been degraded	Use fresh samples or use more samples.
	Elution Buffer amount is not enough.	Be sure 50μl Elution is used to elute the RNA.
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 yield	Inconsistent amounts of magnetic beads	Be sure to vortex the magnetic beads solution before use and vortex beads in the binding step.
	Inconsistent sample is used	Be sure to mix the sample equably before use.
Purity lower than 1.7	The sample is frozen too long time.	If the purity is in the range of 1.7~1.8, the sample can be used for next detection. If the purity is lower than 1.7, don't recommend use such sample to do next detection.

Ⅷ. Composition of Buffers and Solutions

Elution Buffer: DEPC H2O

Ⅸ.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**

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