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JSR Life Sciences MATERIALS INNOVATION

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ExoCap™ Nucleic Acid Elution Buffer

20 assays / Code No. MEX-E

PRODUCT DESCRIPTION

ExoCap™ Nucleic Acid Elution Buffer is optimized to isolate nucleic acids from immunopurified materials, exosomes bound to *ExoCap™ Capture Beads* especially. This reagent is also available for Extracellular vesicles (EVs) research: all of the known EV isolation methods, such as ultracentrifugation, antibody/reagent-based precipitation and size exclusion, can be followed by this *kit*. The extraction procedure requires neither filtration step nor phenol-chloroform extraction step. This method allows us to achieve a high nucleic acids recovery rate with good quality. There are three methods to isolate large RNAs and/or small RNAs. Select the best way which is suitable for your following analysis.

- If the input material contains both RNA and DNA, the nucleic acids isolated by this *kit* could be available for DNA analysis.
- This *kit* is also available for analysis of other nucleic acids-retaining materials.

PRODUCT CONTENTS

1. Nucleic Acid Elution Buffer 1	0.26 mL: enzyme solution
2. Nucleic Acid Elution Buffer 2†	6 mL: diluent for Nucleic Acid Elution Buffer 1
3. Nucleic Acid Elution Buffer 3‡	4 mL: protein dissolvent
4. Nucleic Acid Elution Buffer 4	0.2 mL: co-precipitator

Note: † **Nucleic Acid Elution Buffer 2** may become turbid when stored for long-term at 2-8ºC. Turbidity does not affect performance. If this reagent is turbid, equilibrate to room temperature (15-25ºC) and mix well before use.

‡ Precipitates may appear when **Nucleic Acid Elution Buffer 3** is stored for long-term at 2-8ºC. If this reagent contains precipitates, dissolve them by equilibrating the solution to room temperature (15-25ºC) and mix well before use. This reagent contains guanidine hydrochloride; this is a potentially hazardous substance and should be used with appropriate caution.

STORAGE

ExoCap™ Nucleic Acid Elution Buffer is stable for two years from the date of manufacture when stored at 2-8ºC.

MATERIALS REQUIRED BUT NOT PROVIDED

Equipments

1. Microcentrifuge capable of 12,000 × *g*
2. Microcentrifuge tubes (1.5 mL or 2 mL) (Nuclease-free) [Recommendation; use low-adhesion tube]
3. Pipette tips (10 µL, 20-100 µL, 200 µL, and 1,000 µL) (Nuclease-free) [Recommendation; use low-adhesion pipette tip]
4. Ultra low temperature freezer (-80ºC)
5. Freezer (below -20ºC)
6. Vortex mixer
7. Magnetic stand
8. Gloves

Reagents

9. 100% Ethanol (molecular biology grade)
10. 70% Ethanol (molecular biology grade)
11. 100% 2-Propanol (molecular biology grade)
12. Nuclease-free water

RECOMMENDED PROTOCOLS

- ✧ ***Nucleic Acid Elution Buffer 2*** *and* ***Nucleic Acid Elution Buffer 3*** *should be equilibrated to room temperature before use.*
- ✧ *The reagents should be briefly but thoroughly mixed before use.*
- ✧ *The maximum volume of input EV sample is up to 100 µL.*

§ **Please use one of the three methods described bellow.**

(A) Separation method:

Large RNAs and small RNAs are divided into individual microcentrifuge tubes. By this method, RNAs are split into large and small RNAs based on their length.

(B) 2-step method:

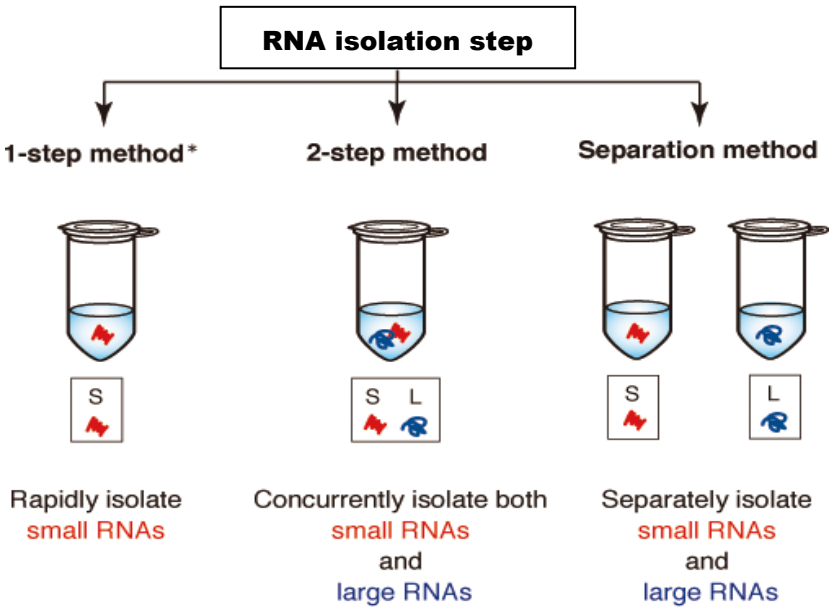
Both large RNAs and small RNAs are simultaneously isolated into one microcentrifuge tube. The advantage of this method is that the recovery rates for both RNAs are higher than the other 2 methods. Please note that the RNAs isolated by this method are not suitable for visualization by silver staining following denaturing urea PAGE because of high background.

(C) 1-step method:

This is a simplified method for isolating small RNAs, but not suitable for isolating large RNAs because the recovery of large RNAs is inefficient. The advantage of this method is that the time required for RNA isolation is short compared with the other 2 methods. Please note that RNAs isolated by this method are mainly small RNAs, while co-purification of large RNAs is observed (~40% of large RNAs).

§ **Comparative table of RNA isolation methods**

	(A) Separation method	(B) 2-step method	(C) 1-step method
Collectable RNA species	large RNA small RNA (in individual tubes)	large RNA small RNA (in one tube)	small RNA (a small amount of large RNA)
Recovery rate for large RNA	>90%	>90%	<40%
Recovery rate for small RNA	>80%	>90%	>90%
Classification by nucleotide length	Yes (large RNA: >60-80 nt) (small RNA: <60-80 nt)	No	No
Assay time	75 min.	75 min.	45 min.
Background (silver staining)	Low	High	Moderate
Advantage	Available for multiple applications	High-recovery rate for large/small RNA	Short assay time
Disadvantage	A little loss in recovery of small RNA compared to the other 2 methods	Not suitable for visualization by silver staining following denaturing PAGE	Low-recovery rate for large RNA (~40% of large RNAs)



*This is not suitable for isolating large RNAs because the recovery for large RNAs is inefficient compared with the other 2 methods.

(A) Separation method

1. Prepare Master mix solution by diluting 10 µL of Nucleic Acid Elution Buffer 1 with 240 µL of Nucleic Acid Elution Buffer 2 per sample.
2. Place the tube, which contains the *ExoCap™ Capture Beads*-bound-EV sample, on a magnetic stand for 1 min and discard the supernatant carefully. Add 250 µL of Master mix solution to each tube, vortex thoroughly and spin-down.

   Note: For beads-free EV samples, which are not immunopurified by antibody-dependent manner, add 250 µL of Master mix solution directly. Please note that each EV sample volume should be less than 100 µL.
3. Add 150 µL of Nucleic Acid Elution Buffer 3 to each tube, vortex thoroughly and spin-down.
4. Dispense 2 µL of Nucleic Acid Elution Buffer 4 to each new microcentrifuge tube for step 6.

   Note: For beads-free EV samples, add 2 µL of Nucleic Acid Elution Buffer 4 to each tube directly, then skip to step 7.
5. Place the tube on a magnetic stand to separate the beads from the solution.
6. After the solution becomes clear (about 1 min), carefully transfer the supernatant to the tube prepared in step 4.
7. Add 300 µL of ice-cold 2-propanol to each tube, vortex briefly but thoroughly, and spin-down.
8. Incubate the tube at -20ºC or below for 20 min (or overnight, if necessary). During the incubation, dispense 2 µL of Nucleic Acid Elution Buffer 4 to each new microcentrifuge tube for step 10.
9. Centrifuge the tube at 12,000 × *g* for 10 min at 4ºC. Large RNAs will be precipitated as a pellet, while small RNAs will remain in the supernatant.
10. Transfer the supernatant, which contains small RNAs, to the tube prepared in step 8. Small RNAs isolation steps from this supernatant are described in the following steps 11–19.

    If small RNAs isolation is not necessary, skip to step 14 and purify large RNAs.

    ✧ **Additional protocol: isolation for small RNAs**

    11. Add 500 µL of ice-cold 2-propanol to the supernatant containing small RNAs prepared in step 10, vortex briefly but thoroughly, and spin-down.
    12. Incubate the tube at -20ºC or below for 20 min (or overnight, if necessary).
    13. Centrifuge the tube at 12,000 × *g* for 10 min at 4ºC, and aspirate the supernatant carefully. The pellet remaining in the tube contains small RNAs.
14. Rinse the pellet with 500 µL of ice-cold 70% ethanol, and mix briefly.
15. Centrifuge the tube at 12,000 × *g* for 3 min at 4ºC, and aspirate the supernatant carefully.
16. Repeat steps 14–15 to rinse the pellet once again.
17. Aspirate the excess ethanol, and leave the tube lids open for 5-15 min at room temperature to evaporate the remaining ethanol. Avoid RNase contamination and overdrying during this step. (Evaporation in clean bench is recommended.)
18. Reconstitute the pellets of the large RNAs and the small RNAs in 20 µL and 10 µL of nuclease-free water, respectively.
19. Store at -80ºC or below until starting following analysis.

(B) 2-step method

1. Prepare Master mix solution by diluting 10 µL of Nucleic Acid Elution Buffer 1 with 240 µL of Nucleic Acid Elution Buffer 2 per sample.
2. Place the tube, which contains the *ExoCap™ Capture Beads*-bound-EV sample, on a magnetic stand for 1 min and discard the supernatant carefully. Add 250 µL of Master mix solution to each tube, vortex thoroughly and spin-down.

   Note: For beads-free EV samples, which are not immunopurified by antibody-dependent manner, add 250 µL of Master mix solution directly. Please note that each EV sample volume should be less than 100 µL.
3. Add 150 µL of Nucleic Acid Elution Buffer 3 to each tube, vortex thoroughly and spin-down.
4. Dispense 2 µL of Nucleic Acid Elution Buffer 4 to each new microcentrifuge tube for step 6.

   Note: For beads-free EV samples, add 2 µL of Nucleic Acid Elution Buffer 4 to each tube directly, then skip to step 7.
5. Place the tube on a magnetic stand to separate the beads from the solution.

6. After the solution becomes clear (about 1 min), carefully transfer the supernatant to the tube prepared in step 4.
7. Add 400 µL of 100% ethanol to each tube, vortex briefly but thoroughly, and spin-down.
8. Incubate the tube at -20ºC or below for 20 min (or overnight, if necessary).
9. Centrifuge the tube at 12,000 × *g* for 10 min at 4ºC, and add 2 µL of Nucleic Acid Elution Buffer 4.
10. Add 400 µL of 100% ethanol to each tube, vortex briefly but thoroughly, and spin-down.
11. Incubate the tube at -20ºC or below for 20 min (or for overnight, if necessary).
12. Centrifuge the tube at 12,000 × *g* for 10 min at 4ºC, and aspirate the supernatant carefully.
13. Rinse the pellet with 500 µL of ice-cold 70% ethanol, and mix briefly.
14. Centrifuge the tube at 12,000 × *g* for 3 min at 4ºC, and aspirate the supernatant carefully.
15. Repeat steps 13–14 to rinse the pellet once again.
16. Aspirate the excess ethanol, and leave the tube lids open for 5-15 min at room temperature to evaporate the remaining ethanol. Avoid RNase contamination and overdrying during this step. (Evaporation in clean bench is recommended.)
17. Reconstitute the pellet in 10 µL of nuclease-free water.
18. Store at -80ºC until starting following analysis.

(C) 1-step method

1. Prepare Master mix solution by diluting 10 µL of Nucleic Acid Elution Buffer 1 with 240 µL of Nucleic Acid Elution Buffer 2 per sample.
2. Place the tube, which contains the *ExoCap*^{TM} *Capture Beads*-bound-EV sample, on a magnetic stand for 1 min and discard the supernatant carefully. Add 250 µL of Master mix solution to each tube, vortex thoroughly and spin-down.
   Note: For beads-free EV samples, which are not immunopurified by antibody-dependent manner, add 250 µL of Master mix solution directly. Please note that each EV sample volume should be less than 100 µL.
3. Add 150 µL of Nucleic Acid Elution Buffer 3 to each tube, vortex thoroughly and spin-down.
4. Dispense 2 µL of Nucleic Acid Elution Buffer 4 to each new microcentrifuge tube for step 6.
   Note: For beads-free EV samples, add 2 µL of Nucleic Acid Elution Buffer 4 to each tube directly, then skip to step 7.
5. Place the tube on a magnetic stand to separate the beads from the solution.
6. After the solution becomes clear (about 1 min), carefully transfer the supernatant to the tube prepared in step 4.
7. Add 800 µL of ice-cold 100% ethanol to each tube, vortex briefly but thoroughly, and spin-down.
8. Incubate the tube at -20ºC or below for 20 min (or for overnight, if necessary).
9. Centrifuge the tube at 12,000 × *g* for 10 min at 4ºC, and aspirate the supernatant carefully.
10. Rinse the pellet with 500 µL of ice-cold 70% ethanol, and mix briefly.
11. Centrifuge the tube at 12,000 × *g* for 3 min at 4ºC, and aspirate the supernatant carefully.
12. Repeat steps 10–11 to rinse the pellet once again.
13. Aspirate the excess ethanol, and leave the tube lids open for 5-15 min at room temperature to evaporate the remaining ethanol. Avoid RNase contamination and overdrying during this step. (Evaporation in clean bench is recommended.)
14. Reconstitute the pellet in 10 µL of nuclease-free water.
15. Store at -80ºC until starting following analysis.

FURTHER ANALYSIS

The isolated EV-derived nucleic acids are available for well-known profiling analysis, such as RT-qPCR, deep sequencing and microarray. Before continuing your analysis, it may be useful to verify the quality of isolated nucleic acids by Bioanalyzer (Agilent Technologies, Inc.) .