T-Pro Biotechnology

T-Pro Total exosome RNA isolation kit

JO67-D001 (50 isolations)

A. Kit Content

T-Pro Total exosome RNA isolation Kit

Catalog No.	**JO67-D001**
Number of preps	**50 isolations**
T-Pro AMR column	**50pcs**
2ml Collection Tube	**100pcs**
Buffer MRL	**12ml**
Buffer RW1 *(Concentrate)	**20ml**
Buffer RW2* (Concentrate)	**8ml**
Nuclease-Free Water	**10ml**
Proteinase K Buffer	**1.6 ml**

⚠ Buffer MRL and RW1 contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach.

⚠ **Before use**

1. Add 20ml ethanol (96-100%) to RW1 (total final volume:40ml)
2. Add 32ml ethanol (96-100%) to RW2 before use.(total final volume:40ml)
3. After receiving the kit, store it the Proteinase K at 4°C

B. Storage and Stability

- The kit can be ship and store at +2 to +30 °C (+35.6 to +86 °F) for 30 months
- The kit components are stable until the expiration date printed on the label

C. Intended Use

- The T-Pro Total Exosome RNA Isolation Kit is designed for isolation of RNA from a single enriched exosome preparation.
- Maximal yields of ultra-pure RNA can be prepared in about 30 minutes, and are suitable for studies of RNA expression (specifically miRNA), processing, or function. The RNA can be used in downstream application, including RT-PCR, sequencing (e.g., Ion PGM™, Ion Proton™, or SOLiD® Systems), RNA amplification, microarray analyses, solution hybridization assays, and blot hybridization.

D. Sample Type

- This kit is suitable for extraction exosome RNA (microRNA; miRNA) from enriched exosome samples, serum, plasma, liquid transport media (e.g. UTM),culture media or clear cell-free body fluid
- Sample input: 200ul
- Using T-Pro Total Exosome isolation reagents to collect the exosomes from cell culture media or serum is recommended:

  A. Reagent for cell culture media: (JO66-V001S) 100ml/(JO66-V001M) 500ml

  B. Reagent for serum: (JO66-V0025) 5x1ml /(JO66-V002M) 25ml

⚠ Treat all samples as potentially infectious, follow the local safety regulation

⚠ The sample must be chilled immediately on wet ice and the nucleic acid isolated within four hours after collection

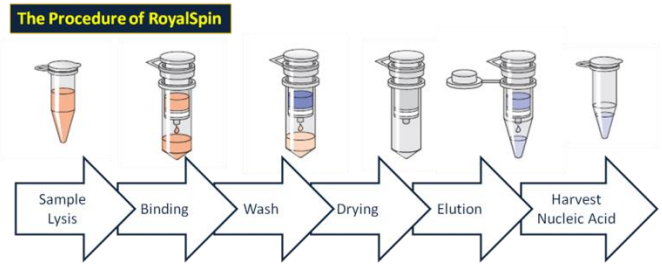
⚠ If the plasma/ serum sample can't be processing immediate, should be frozen after remove red blood cells contamination. The frozen sample should be shipped to the laboratory on dry ice

E. Additional Equipment and Reagents Required

1. Standard laboratory equipment
2. Pipettes

3. Nuclease-free, aerosol-preventive filter tips
4. Ethanol (96–100%) or isopropanol (highly recommended)
5. 1.5ml microcentrifuge tubes
6. Disposable gloves
7. Heating block for lysis of samples at 56°C
8. Microcentrifuge (with rotor for 1.5ml and 2ml tubes)
9. Vortex mixer
10. For samples <200µl: 0.9% NaCl solution or PBS
11. T-ProTotal Exosome isolation reagents (cell culture media)
12. T-Pro Total Exosome isolation reagents (serum)

F. Protocol of Purification



This protocol is for purification of exosome RNA from plasma, serum, cell culture media or other cell-free body fluid using the T-Pro total Exosome RNA isolation Kit and a microcentrifuge.

Important point before starting

All centrifugation steps are carried out at room temperature (15–25°C).

Things to do before starting

1. Equilibrate frozen samples, place it on ice
2. Equilibrate Buffer MRL, RW1,RW2 and Nuclease-free water to room temperature
3. Turn on heating block(or thermomixer) and set the temperature at 56°C heating block
4. Ensure that **Buffer RW1 and Buffer RW2** have been prepared according to the description on section A

G. Procedure

1. **Pipet 30µl proteinase buffer and 200µl sample into a 1.5ml microcentrifuge tube**
   - If the sample volume is less than 200µl, add the appropriate volume of 0.9% NaCl solution to bring the volume of protease and sample up to a total of 230µl
2. **Add 200µl Buffer MRL to sample mixture, close the cap and mix by pulse-vortexing for 15sec**
   - In order to ensure efficient lysis, it is essential that the sample and Buffer MRL are mixed thoroughly to yield a homogeneous solution
3. **Incubate at 56°C for 10-15min in a heating block**
4. **Briefly centrifuge the 1.5ml tube to remove drops from the inside of the lid**
5. **Add 600µl isopropanol (3X volume of sample) or 250µl absolute ethanol (1.25 X of sample) to the sample, close the cap and mix thoroughly by pulse-vortexing for 15sec. Incubate the lysate for 2min at room temperature (15–25°C)**
   - If ambient temperature exceeds 25°C, ethanol or isopropanol should be cooled on ice before adding to the lysate
   - Isopropanol is recommended
6. **Briefly centrifuge the 1.5ml tube to remove drops from the inside of the lid**
7. **Put T-Pro AMR column into a 2ml collection tube. Carefully apply all of the lysate mixture onto the column without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the flow- through**
   - If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the column is empty
8. **Carefully open the T-Pro AMR column, and add 500µl of Buffer RW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the flow- through**
9. **Carefuly open the T-Pro AMR column, and add 600µl of Buffer RW2 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min.**

Discard the flow- through

10. **(Optional) Carefully open the T-Pro AMR column, and add 600µl of ethanol without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the flow- through**
11. **Place the T-Pro AMR column into a new 2ml collection tube, Close the cap and Centrifuge at full speed (12,000 x g; 14,000 rpm) for 3 min**
12. **(Optional) Place the T-Pro AMR Column into a new 2ml collection tube (not provided), open the lid, and incubate the assembly at 56°C for 3 min to dry the membrane completely.**
13. **Place the T-Pro AMR column in a clean 1.5ml microcentrifuge tube (not provided), and discard the collection tube with the filtrate. Carefully open the lid of the column, and apply 20–150µl of RNase-free water to the center of the membrane. Close the lid and incubate at room temperature for at least 2 min. Centrifuge at full speed (12,000 x g; 14,000 rpm) for 1 min**
14. **(Optional) Repeat the elution once more with an addition alquot of 50 µL of nuclease-free water.**
15. **Collect the eluate (which contains the RNA) and place on ice for immediate use, or store it at ≤ –20°C.**

✍ **Important:** The nuclease-free water must be dispensed onto the center of the membrane for complete elution of bound nucleic acid. Elution volume is flexible and can be adapted according to the requirements of the downstream application. Remember that the recovered eluate volume will be approximately 5 µl less than the elution buffer volume applied onto the column.

H. Order Information

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Cat. No.	Product name	Spec.
JO67-D001	T-Pro Total Exosome RNA Isolation Kit	50 preps
JO66-V0015	T-Pro Total Exsome Isolation Reagent(for culture media)	100ml
JO66-V001M	T-Pro Total Exsome Isolation Reagent(for culture media)	500ml
JO66-V0025	T-Pro Total Exsome Isolation Reagent(for serum)	5 x1ml
JO66-V002M	T-Pro Total Exsome Isolation Reagent(for serum)	25ml