VitroSure™ RNA FFPE Tissue Isolation Kit
(RTU for 50 Preparations)

SKU: VB-5002

Description

VitroSure™ RNA FFPE Tissue Isolation Kit is a high-performance solution designed specifically for extracting high-quality RNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples. Ideal for small sample volumes, this kit utilizes trusted VitroSure RNA Elute Columns and a silica-based membrane technology to ensure efficient purification of total RNA. With flexible elution volumes ranging from 15–30 µl, the kit supports downstream applications such as RNA-seq, RT-PCR, qPCR, and gene expression analysis, delivering reliable results.

Technical Specifications

Equipment needed	Microcentrifuge, heat block/bath (37°C, 56°C, and 80°C)
RNA Type Isolated	Total RNA
Size Range	> 70 bp
Yield	Up to 25 g total RNA can be eluted into ≥ 20 µl
Purity	Typical A260/A280 ≥ 1.8
Eluted RNA Storage	at ≤ -20°C
Sample Source	Tissue from a paraffin block or FFPE tissue sections
Processing Capacity	FFPE Tissue: ≤25 mg or 2-8 sections at a thickness of 7-10 µm with a surface area of 15-20 mm^2
Applicable For	RT-PCR, hybridization and Next generation sequencing (NGS), etc.

Kit Contents

Components	Volume
Buffer VKD	10 ml
Buffer VLT	20 ml
Buffer VRN	50 ml
Buffer VDD	5 ml
Buffer VPE	30 ml
DNase I Powder	1 mg
Proteinase K Powder	5 mgx3
Proteinase K Buffer	1 ml
RNase Free Water	1 mlx2
VitroSure RNA Elute Columns	50
Collection Tubes (2ml)	100

Storage

Store the Proteinase K Powder and DNase I powder at -20°C. After reconstitution of Proteinase K and DNase I store the solution at -20°C. The rest can be stored at room temperature.

Procedures

1. Sample preparation
   1) Sample preparation from FFPE block using the Deparaffinization Solution (VB-9008, sold separately):
      a. Use a scalpel to trim excess paraffin off the sample block.
      b. Cut up to 2-6 sections with a microtome, each 5–10 µm thick. The section number for each sample depends on the tissue size. Discard the first 2–3 sections if the sample surface has been exposed to air.
      c. Place the sections immediately in a 1.5 or 2 ml microcentrifuge tube
      d. Add Deparaffinization Solution: for 2-6 sections or one 20 µm section, add 320 µl Deparaffinization Solution; for more sample material, add 640 µl Deparaffinization Solution.
      e. Vortex vigorously for 10 s, and centrifuge briefly to bring the sample to the bottom of the tube.
      f. Incubate at 56°C for 3 min, then allow to cool at room temperature (15–25°C), and centrifuge at full speed for 2 min.
      g. Carefully remove the supernatant by pipetting without disturbing the pellet. Carefully remove any residual Deparaffinization Solution using a fine pipette tip.
      h. Keep the lid open and incubate for 10 min at 37°C to dry the pellet. Proceed to step 3
   2) Sample preparation from FFPE block using Xylene:
      a. Use a scalpel to trim excess paraffin off the sample block.
      b. Cut up to 2-6 sections with a microtome, each 5–10 µm thick. The section number for each sample depends on the tissue size. Discard the first 2–3 sections if the sample surface has been exposed to air.
      c. Place the sections immediately in a 1.5 or 2 ml microcentrifuge tube and add 1 ml of xylene to the sample. Close the lid and vortex vigorously for 10 seconds.
      d. Centrifuge at maximum speed for 2 minutes at room temperature.
      e. Carefully remove the supernatant without disturbing the pellets.
      f. Add 1 ml of ethanol (96–100%) to the pellet and mix by vortexing to extract residual xylene from the sample.
      g. Centrifuge at maximum speed for 2 minutes at room temperature.
      h. Carefully remove the supernatant without disturbing the pellet. Remove any remaining ethanol with a fine pipette tip.
      i. Open the tube and incubate at room temperature or up to 37°C for 10minutes or until all residual ethanol has evaporated. Proceed to step 3
   3) Sample preparation from FFPE sections on slides:
      a. Submerge the slides in xylene I for 3 minutes, followed by xylene II for an additional 3minutes.
      b. Remove xylene by rinsing with 100% ethanol (1 minute each, repeated twice).
      c. Air dry the slides for 3-5minutes.
      d. Gently detach the tissue sections from the slides using a small blade, then transfer the tissue pellets into a 1.5 ml microcentrifuge tube. Proceed to step 3

2. Reconstitute the Proteinase K solution by combining 260 µl of Proteinase K buffer with 5 mg of Proteinase K powder. For the DNase I solution, add 550 µl of RNase-Free Water to 1 mg of DNase I powder, and gently mix by inverting the vial. After reconstitution, aliquot the Proteinase K and DNase I solutions, then store them at -20°C. Please refrain from subjecting the solutions to repeated thaw-and-melt cycles to maintain their stability.
3. Resuspend the pellets in 150 µl Buffer VKD. Add 10 µl of proteinase K solution and mix by vortexing.
4. Incubate at 56°C for 15 min and then incubate on ice for 3 min.
5. Centrifuge for 15 min at 20,000×g and carefully transfer the supernatant to a new microcentrifuge tube without disturbing the pellet.
6. Incubate at 80°C for 15 min.
7. Add 320 µl of Buffer VLT to the sample and mix thoroughly byvortexing.
8. Add 720 µl ethanol (96–100%) and mix well by vortexing or pipetting.
9. Carefully transfer the entire lysate to an RNA Elute column and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through. Repeat this step until the complete sample is used.
10. Add 350 µl of Buffer VRN and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through.
11. Mix 10 µl DNase I solution with 70 µl Buffer VDD gently and add directly to the column membrane. Incubate at 20–30°C for 15 min.
12. Add 500 µl of Buffer VRN and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Save the flow-through.
13. Place the column in a clean 2 ml collection tube. Apply the flow-through to the column and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through.
14. Add 500 µl Buffer VPE to the column. Centrifuge at 8000×g (or 10000 rpm) for 1 minute and discard the flow-through. Repeat this step once more.
15. Centrifuge at maximum speed for 3 minutes with the lid open to completely dry the membrane.
16. Place the column in a clean 1.5 ml microcentrifuge tube and apply 10–30 µl RNase-free water to the center of the membrane. Ensure that RNase-free water is at room temperature.
17. Incubate for 1 min at room temperature (15–25°C) and centrifuge at full speed (20,000×g or 14,000 rpm) for 1 min to elute the RNA.

Note: This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Precautions: Handle with care. Avoid contact with eyes, skin and clothing. Do not ingest. Wear gloves.