VitroVivo Biotech
Make Tissues, Cells, Genes and Molecules Visible

VitroSure™ RNA FFPE Tissue Isolation Kit
(For 20 Preps)

SKU#: VB-5002s

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 with 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 µg total RNA can be eluted into ≥ 20 µl
Purity	Typical A260/A280 ≥ 1.8
Eluted RNA Storage	at ≤ -20°C
Sample Source	Tissue from paraffin block or FFPE tissue sections
Processing Capacity	FFPE Tissue : ≤25 mg or 2-8 sections at 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:

Buffer VKD	5 ml
Buffer VLT	10 ml
Buffer VRN	20 ml
Buffer VDD	2 ml
Buffer VPE	30 ml
DNase I Powder	1 mg
Proteinase K Powder	5 mg
Proteinase K Buffer	0.5 ml
RNase Free Water	1 ml
VitroSure RNA Elute Columns	20
Collection Tubes (2ml)	40

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:
        a. Use a scalpel to trim excess paraffin off the sample block.
        b. Cut up to 8 sections, each 5–10 µm thick. 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 10 minutes or until all residual ethanol has evaporated.
    2) Sample preparation from FFPE sections on slides:
        a. Submerge the slides in xylene I for 3 minutes, followed by xylene II for an additional 3 minutes.
        b. Remove xylene by rinsing with 100% ethanol (1 minutes each, repeated twice).
        c. Air dry the slides for 3-5 minutes.
        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.
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 by vortexing.
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 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.