VitroVivo Biotech
Make Tissues, Cells, Genes and Molecules Visible

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

SKU#: VB-5003s

Description:
VitroSure™ DNA/RNA FFPE Tissue Isolation Kit is specially designed to optimize for the simultaneous isolation genomic DNA and total RNA (including small RNAs) from FFPE tissue sections. Pure DNA and RNA are released sequentially by differential solubilization of the same FFPE entire sample.

Kit Contents:

Buffer VKD	5 ml
Buffer VLT	10 ml
Buffer VRN	20 ml
Buffer VDD	2 ml
Buffer VPE	30 ml
Buffer VTL	5 ml
Buffer VL	5 ml
Buffer VW1	15 ml
Buffer VW2	15 ml
Buffer VTE	2 ml
RNase A (100mg/ml)	50 µl
DNase I Powder	1 mg
Proteinase K Powder	5 mg×3
Proteinase K Buffer	1 ml
RNase Free Water	1 ml
VitroSure DNA Elute Columns	20
VitroSure RNA Elute Columns	20
Collection Tubes (2ml)	80

Technical Specifications:

Equipment needed	Microcentrifuge, heat block/bath (37°C, 56°C, 80°C and 90°C)
Type Isolated	Total DNA/RNA
Size Range	DNA> 50 bp/RNA> 70 bp
Yield	Up to 25 µg total DNA/RNA can be eluted into ≥ 50 µl/≥ 20 µl
Purity	Typical A260/A280 ≥ 2.0
Eluted RNA Storage	at ≤ -20°C
Sample Source	Tissue from paraffin block or 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	PCR and Next generation sequencing (NGS), genotyping, Restriction enzyme digestion, SNP, etc.

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 for RNA purification.
6. Keep the pellet for DNA purification.

RNA Purification

7. Incubate the supernatant from step 5 at 80°C for 15 min.
8. Add 320 μl of Buffer VLT to the sample and mix thoroughly by vortexing.
9. Add 720 μl ethanol (96–100%) and mix well by vortexing or pipetting.
10. 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.

11. Add 350 µl of Buffer VRN and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through.
12. Mix 10 µl DNase I stock solution with 70 µl Buffer VDD gently and add directly to the column membrane. Incubate at 20–30°C for 15 min.
13. Add 500 µl of Buffer VRN and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Save the flow-through.
14. 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.
15. 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.
16. Centrifuge at maximum speed for 3 minutes with the lid open to completely dry the membrane.
17. 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.
18. 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.

DNA Purification

19. Resuspend the pellet from step 6 in 180 µl Buffer VTL. Add 20 µl proteinase K solution and mix by vortexing.
20. Incubate at 56°C for 1 h and then incubate at 90°C for 2 h without agitation.
21. Cool down to room temperature. Add 2 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature if RNA-free genomic DNA is required.
22. Add 200 µl Buffer VL and 200 µl ethanol to the sample. Mix thoroughly by vortexing.
23. Transfer the entire sample to a DNA Elute column and centrifuge at 8000×g (or 10000 rpm) for 1 min. Discard the flow-through.
24. Add 500 µl of Buffer VW1 and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through.
25. Add 500 µl of Buffer VW2 and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through and collection tube.
26. Place the DNA Elute column in a clean 2 ml collection tube. Centrifuge at maximum speed for 3 minutes with the lid open to completely dry the membrane.
27. Place the DNA Elute column in a clean 1.5 ml microcentrifuge tube and apply 20–100 µl of Buffer VTE to the center of the membrane. Ensure that Buffer VTE is at room temperature.

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.