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

SKU#: VB-5003s

Description:

VitroSure™ DNA/RNA FFPE Tissue Isolation Kit is engineered to efficiently isolate high-quality genomic DNA and total RNA—including small RNAs—from formalin-fixed, paraffin-embedded (FFPE) tissue sections. This all-in-one kit enables sequential extraction of pure DNA and RNA from the same entire sample through a differential solubilization process, maximizing sample utility and preserving nucleic acid integrity for downstream applications such as NGS, RT-PCR, and gene expression analysis.

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 DNA: A260/A280 ≥ 1.8 /RNA: 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.

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

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.