VitroSure™ DNA/RNA FFPE Tissue Isolation Kit
(For 50 Preps)

SKU: VB-5003

Description:

The VitroSure™ DNA/RNA FFPE Tissue Isolation Kit enables consistent extraction of high-quality genomic DNA and total RNA—including small RNAs—from tissue sections preserved with formalin and embedded in paraffin. 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 a paraffin block or 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	PCR and Next Generation Sequencing (NGS), genotyping, Restriction enzyme digestion, SNP, 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
Buffer VTL	15 ml
Buffer VL	15 ml
Buffer VDW1	30 ml
Buffer VDW2	30 ml
Buffer VTE	6 ml
RNase A (100mg/ml)	120 µl
DNase I Powder	1 mg

Proteinase K Powder	5 mg×8
Proteinase K Buffer	3 ml
RNase Free Water	2 ml
VitroSure DNA Elute Columns	50
VitroSure RNA Elute Columns	50
Collection Tubes(2ml)	200

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-5009):
      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 (VB-5009): 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 is 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 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 Buffer VLT to the sample and vortex to mix.
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 the 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. Add500µlofBufferVRN andcentrifugeat8000×g (or10000 rpm) for1 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. Placethecolumninaclean1.5ml microcentrifugetubeandapply10–30µl 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. If RNA-free genomic DNA is required, add 2 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature.
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 VDW1 and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard

the flow-through.

25. Add 500 µl of Buffer VDW2 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.