



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

SKU: VB-5003s

**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 <sup>2</sup>
Applicable For	PCR and Next Generation Sequencing (NGS), genotyping, Restriction enzyme digestion, SNP, etc.

**Kit Contents:**

<b>Components</b>	<b>Volume</b>
Buffer VKD	5ml
Buffer VLT	10 ml
Buffer VRN	20 ml
Buffer VDD	2ml
Buffer VPE	15ml
Buffer VTL	5 ml
Buffer VL	5 ml
Buffer VDW1	15 ml
Buffer VDW2	15 ml
Buffer VTE	2 ml
RNase A (100mg/ml)	50 µl
DNase I Powder	1mg

Proteinase K Powder	5 mg×3
Proteinase K Buffer	1ml
RNase Free Water	1ml
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 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 10 minutes 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 3 minutes.

- b. Remove xylene by rinsing with 100% ethanol (1 minute 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. Proceed to step 3.
2. Reconstitute the Proteinase K solution by combining 260  $\mu$ l of Proteinase K buffer with 5 mg of Proteinase K powder. For the DNase I solution, add 550  $\mu$ l 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  $\mu$ l Buffer VKD. Add 10  $\mu$ l 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 $\times$ 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  $\mu$ l Buffer VLT to the sample and vortex to mix.
9. Add 720  $\mu$ l 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 $\times$ g (or 10000 rpm) for 1 minute. Discard the flow-through. Repeat this step until the complete sample is used.
11. Add 350  $\mu$ l of Buffer VRN and centrifuge at 8000 $\times$ g (or 10000 rpm) for 1 minute. Discard the flow-through.
12. Mix 10  $\mu$ l DNase I stock solution with 70  $\mu$ l Buffer VDD gently and add directly to the column membrane. Incubate at 20–30°C for 15 min.
13. Add 500  $\mu$ l of Buffer VRN and centrifuge at 8000 $\times$ 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 $\times$ g (or 10000 rpm) for 1 minute. Discard the flow-through.
15. Add 500  $\mu$ l Buffer VPE to the column. Centrifuge at 8000 $\times$ 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  $\mu$ l 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 $\times$ g or 14,000 rpm) for 1 min to elute the RNA.

### DNA Purification

19. Resuspend the pellet from step 6 in 180  $\mu$ l Buffer VTL. Add 20  $\mu$ l 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  $\mu$ l RNase A (100 mg/ml) and incubate for 2 min at room temperature.
22. Add 200  $\mu$ l Buffer VL and 200  $\mu$ l ethanol to the sample. Mix thoroughly by vortexing.
23. Transfer the entire sample to a DNA Elute column and centrifuge at 8000 $\times$ g (or 10000 rpm) for 1 min. Discard the flow-through.
24. Add 500  $\mu$ l of Buffer VDW1 and centrifuge at 8000 $\times$ g (or 10000 rpm) for 1 minute. Discard

- the flow-through.
25. Add 500  $\mu$ l of Buffer VDW2 and centrifuge at 8000 $\times$ 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  $\mu$ l 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.