

VitroSure[™] DNA FFPE Tissue Isolation Kit (For 50 Preps)

SKU#: VB-5001

Description:

VitroSure[™] DNA FFPE Tissue Isolation Kit is optimized for isolation DNA from FFPE tissue samples and uses well-established VitroSure DNA Elute Columns for purification of genomic and mitochondrial DNA from small sample volume size. This kit combines the selective binding properties of a silica-based membrane with flexible elution volumes of 20-100 µl.

Kit Contents:

Buffer VTL	15 ml
RNase A (100 mg/ml)	120 µl
Buffer VL	15 ml
Buffer VW1	30 ml
Buffer VW2	30 ml
Buffer VTE	6 ml
Proteinase K Powder	5 mg×4
Proteinase K Buffer	2 ml
VitroSure DNA Elute Columns	50
Collection Tubes (2ml)	100

Technical Specifications:

Equipment needed	Microcentrifuge, heat block/bath (37°C, 56°C and 90°C)
DNA Type Isolated	Total DNA
Size Range	> 50 bp
Yield	Up to 25 μg total DNA can be eluted into ≥ 50 μl
Purity	Typical A260/A280 ≥ 1.8
Eluted DNA Storage	at ≤ -20°C
Sample Source	Tissue from paraffin block or tissue sections
Processing Capacity	FFPE Tissue : \leq 25 mg or 2-8 sections at thickness of 7-10 μ m 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 RNase A (100 mg/ml) at 2-8°C. After reconstitution of Proteinase K, 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 $5-10 \mu m$ 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, followedby 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. Proteinase K solution preparation: Combine 260 μl of Proteinase K Buffer with 5 mg of Proteinase K Powder. Vortex the mixture to ensure complete dissolution. Store the solution at -20°C.
- 3. Resuspend the pellets in 180 μl Buffer VTL. Add 20 μl of proteinase K solution and mix by vortexing.
- 4. Incubate at 56°C for 1 hour or until the sample is completely lysed.
- 5. Incubate at 90°C for 1 hour without agitation.
- 6. Cool to room temperature and briefly centrifuge the tube. For RNA-free genomic DNA, add 2 μ l of RNase A (100 mg/ml) and incubate for 2 minutes at room temperature.
- 7. Add 200 μ l of Buffer VL and 200 μ l ethanol to the sample. Mix thoroughly by vortexing.
- 8. Carefully transfer the entire lysate to a DNA Elute column and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through.
- 9. Add 500 μl of Buffer VW1 and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through.
- 10. Add 500 μ l of Buffer VW2 and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through and collection tube.
- 11. 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.
- 12. Place the DNA Elute column in a clean 1.5 ml microcentrifuge tube and apply 20–100 μ l of Buffer VTE to the center of the membrane. Ensure that Buffer VTE is at room temperature.

13. Incubate at room temperature for 5 minutes and centrifuge at maximum speed (20,000×g or 14,000 rpm) for 1 minute to elute the DNA.

Note: This product is intended for research purposes only. This product is <u>not</u> 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.