VitroSure™ DNA Isolation Kit for Frozen and Fresh Cells & Tissues

(RTU for 50 Preparations)

SKU:VB-5004

Product Description

The VitroSure™ DNA Isolation Kit is designed for efficient purification of high-quality genomic DNA from frozen or fresh cells and tissue samples. Utilizing the selective binding properties of the VitroSure™ Resin Column, this kit provides a rapid, reliable, and user-friendly workflow. Up to 100 µg of highly purified genomic DNA can be isolated per preparation, suitable for a wide range of downstream molecular biology applications.

Technical Specifications

Parameter	Specification
Required Equipment	Microcentrifuge, heat block or water bath (56 °C)
DNA Type	Genomic DNA
Size Range	15–30 kb
Yield	Up to 100 µg total DNA (eluted in ≥50 µL)
Purity	Typical A260/A280 ≥ 1.8
Storage of Eluted DNA	≤ −20 °C
Sample Types	Frozen or fresh mammalian tissues, cell pellets
Input Capacity	100 cells to 1 × 10^7 cells; ≤30 mg tissue
Downstream Applications	PCR, NGS, genotyping, restriction digestion, SNP analysis

Kit Contents

Components	Quantity
Proteinase K Powder	5 mg × 4
Proteinase K Buffer	1.5 mL
Buffer VTL	15 mL
Buffer VL	15 mL
Buffer VDW1	30 mL
Buffer VDW2	30 mL
Buffer VTE	10 mL
VitroSure™ DNA Elute Columns	50
Collection Tubes (2 mL)	100

Storage Conditions

- Store **Proteinase K** powder and reconstituted Proteinase K at**−**20 °C**.**
- All remaining components may be stored at room temperature**.**

Before Starting

Proteinase K Solution Preparation

- Add 260 µL Proteinase K Buffer to 5 mg Proteinase K powder**.**
- Vortex thoroughly until fully dissolved.
- Store the prepared solution at −20 °C**.**

Protocol 1: Genomic DNA Purification from Mammalian Tissue and Mouse/Rat Tail

1. Sample Preparation and Digestion

Recommended Sample Amounts

- ≤30 mg minced mammalian tissue
- ≤10 mg spleen tissue
- Mouse tail: ≤1 cm
- Rat tail: ≤0.5 cm

Tissue Disruption Recommendations

- Finely mince tissue prior to lysis.
- Grind in liquid nitrogen before adding Buffer VTL and Proteinase K.
- Homogenize tissue for improved lysis efficiency.
- For OCT-embedded tissue, section at 10–15 µm using a cryostat and remove OCT with PBS.
- For adult rodents, use 0.4–0.6 cm tail segments for optimal results.

2. Lysis

- Add 180 µL Buffer VTL and 20 µL Proteinase K. Ensure the tissue is fully submerged.
- Vortex thoroughly.
- Incubate at 56 °C for 1–4 hours, or until completely lysed. Vortex occasionally. Overnight digestion is recommended for tail samples or dense tissues.
- Centrifuge at maximum speed for 3 minutes. Transfer the supernatant to a new tube.

3. RNA Removal (Option)
If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml) (SKU# VB-5011**,** sold separately), vortex to mix, and incubate at room temperature for 2 min, then continue with step 4.

4. DNA Binding

- Add 200 µL Buffer VL**,** vortex for 15 seconds.
- Add 200 µL ethanol (96–100%)**,** vortex thoroughly.
- Transfer the entire lysate to a DNA Elute Column and centrifuge at 8000 × g (≈10,000 rpm) for 1 minute**.** Discard flow-through.

5. Column Washing

- Add 500 µL Buffer VW1**,** centrifuge 1 minute; discard flow-through.
- Add 500 µL Buffer VW2, centrifuge 1 minute; discard flow-through and collection tube.

6. Membrane Drying

- Place column in a clean 2 mL tube and centrifuge at maximum speed for 3 minutes with the lid open.

7. DNA Elution

- Transfer column to a clean 1.5 mL tube.
- Apply 20–100 µL Buffer VTE to the membrane center.
- Incubate for 1 minute at room temperature, then centrifuge at ≥20000 × g (≈14000 rpm) for 1 minute**.**

Note: Lower elution volumes increase DNA concentration but reduce total yield.

Protocol 2: Genomic DNA Purification from Blood, PBMCs, or Cultured Cells

1. Sample Preparation

- **Blood with non-nucleated erythrocytes:**
  Add 20 µL Proteinase K to 50-100 µL blood and adjust to 220 µL with PBS.
- **Blood with nucleated erythrocytes:**
  Add 20 µL Proteinase K into 10~50 µL blood; adjust to 220 µL with PBS.
- **Cultured cells or PBMCs:**
  Pellet ≤5 × 10^6 cells, resuspend in 200 µL PBS, and add 20 µL Proteinase K.
- **Frozen cell pellets:**
  Thaw partially and add PBS until the pellet disperses easily. Resuspend in 200 µL PBS, add 20 µL Proteinase K.

Note: For highly polyploid cells (e.g., HeLa), use fewer cells than the maximum recommended.

2. RNA Removal (Optional)

If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml), vortex to mix, and incubate at room temperature for 2 min, then continue with step 3.

3. Lysis

- Add 200 µL Buffer VL, vortex thoroughly, and incubate at 56 °C for 10 minutes.

4. DNA Binding

- Add 200 µL ethanol (96–100%)**,** vortex thoroughly.
- Transfer lysate to DNA Elute Column and centrifuge at 8000 × g for 1 minute**.**

5. Washing and Elution

- Wash with 500 µL Buffer VW1, then 500 µL Buffer VW2, centrifuging 1 minute each.
- Dry membrane by centrifugation at maximum speed for 3 minutes**.**
- Elute DNA with 20–100 µL Buffer VTE as described in Protocol 1.

Regulatory Statement

For research use only (RUO)**.** Not intended for diagnostic or therapeutic use in humans or animals.

Safety Precautions

- Avoid contact with eyes, skin, and clothing.
- Do not ingest.
- Wear appropriate personal protective equipment, including gloves.