VitroSure™ DNA/RNA Isolation Kit for Frozen, Fresh Cell and Tissue
(For 20 Preps)

SKU: VB-5006s

Description:

The isolation of high-quality, intact DNA and RNA is a critical prerequisite for downstream molecular biology applications, including next-generation sequencing (NGS), Quantitative PCR (qPCR), microarray analysis, and Southern/Northern blotting. The integrity and purity of extracted nucleic acids directly determine the accuracy, sensitivity, and reproducibility of these essays.
The VitroSure™ DNA/RNA Isolation Kit is designed for rapid, efficient, and simultaneous purification of total DNA and total RNA from a broad range of challenging biological sample types. The kit enables reliable recovery of high-quality nucleic acids while preserving their integrity, ensuring consistent performance in demanding downstream applications.

Kit Contents:

Components	Volume
Proteinase K Powder	5mg×2
Proteinase K Buffer	1ml
Buffer VLT	15 ml
Buffer VRW1	15 ml
Buffer VPE	15 ml
Buffer VDW1	15 ml
Buffer VDW2	15 ml
Buffer VTE	3 ml
RNase-Free Water	1.5 ml
VitroSure DNA Elute Columns	20
VitroSure RNA Elute Columns	20
Collection Tubes (2ml)	80

Storage

Store at room temperature.

Procedures

1. Sample Preparation:
   For fresh, frozen cells
   For cultured cells (Recommended from cell culture no more than 1×10^7 cells)
   1) Cells grown in suspension culture:
      a. Determine the number of cells and pellet the cells by centrifuging for 5 min at 300×g in a centrifuge tube.

b. Carefully remove all supernatant by aspiration and proceed to purification step 2.

2) Cells grown in adherent culture:

a. Determine the number of cells and aspirate the medium.
b. Wash the cells with PBS and aspirate the PBS.
c. Add 0.1–0.25% trypsin in PBS.
d. After the cells detached from the dish or flask, add medium, transfer the cells to an
e. RNase-free glass or polypropylene centrifuge tube.
f. Centrifuge at 300×g for 5 min.
g. Completely aspirate the supernatant and proceed to purification step 2.

For blood cells (Recommended from leukocyte cells from 5 x 10^6/ml to 1 x 10^7/ml)

a. Pellet the cells by centrifugation at 1000 x g for 15 min.
b. Resuspend the sample in 1 ml of PBS.
c. Completely aspirate the supernatant and proceed to purification step 2.

If the sample is frozen cells, thaw the cells and spin them down. Completely aspirate the Supernatant and proceed to purification step 2.

For mammalian tissue

1) Place the following amount of mammalian tissue directly into a suitably sized vessel for disruption and homogenization:

- ≤30 mg of minced mammalian tissue
- ≤10 mg minced spleen tissue

2) Proceed to purification step 2.

2. Proteinase K treatment (optional) can be performed on protein-rich samples stored (e.g., tissue, blood cells, etc.), in Buffer VKD (VB-5010, sold separately) using Proteinase K:
    - Reconstitute the Proteinase K solution by combining 260 µl of Proteinase K buffer with 5 mg of Proteinase K powder. After reconstitution, aliquot the Proteinase K, then store them at -20°C. Please refrain from subjecting the solutions to repeated thaw-and-melt cycles to maintain their stability.
    - Resuspend the pellets in 80 µl Buffer VKD. Add 10 µl of proteinase K solution and mix by vortexing.
    - Incubate at 56°C for 15 min
3. Resuspend the pellets or tissue in 600 µl Buffer VLT and homogenize the lysate with a suitable homogenizer for 2 minutes. Note: Add 10 µl β-ME per 1 ml Buffer VLT before use.
4. Transfer all the lysate to a DNA Elute column placed in a 2 ml collection tube and centrifuge for 30 s at full speed (maximum 20,000×g).
5. Place the DNA Elute column in a new 2 ml collection tube and store at 2–8°C for the DNA purification step.

RNA Purification

6. Transfer the flow-through to a new 2 ml microcentrifuge tube.
7. Add 1 volume of 70% ethanol to the homogenized lysate and mix well by pipetting.
8. Transfer all the lysate to an RNA Elute column and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through.
9. Add 350 µl Buffer VRW1 to the column and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through.
10. Eliminating Genomic DNA Contamination (Optional): DNase digestion is generally not required, as silica-membrane technology effectively removes most genomic DNA. However, for applications highly sensitive to trace DNA contamination, an additional DNase treatment using DNase set (SKU# VB-5007, sold separately) is recommended.
    - For the preparation of DNase I stock 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 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.

- Add 10 µl DNase I stock solution to 70 µl Buffer VDD. Mix by gently inverting the tube.
- Add the above DNase I solution directly to the column membrane and incubate at RT (20–30°C) for 15 min.

11. Add 350 µl Buffer VRW1 to the column and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through.
12. Add 500 µl Buffer VPE to the column and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through. Repeat this step.
13. Place the column in a clean 2 ml collection tube. Centrifuge at maximum speed for 3 minutes with the lid open to completely dry the membrane.
14. Place the column in a new 1.5 ml collection tube and add 30-50 µl RNase-free water directly to the column membrane. Incubate at room temperature for 1 min, then centrifuge at maximum speed for 1 min to elute the RNA.

DNA Purification

15. Add 350 µl Buffer VDW1 to the DNA Elute column from step 4 and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through.
16. Gently mix 20 µl Proteinase K with 60 µl Buffer VDW1 and apply to the column membrane and incubate for 5 min at room temperature.
17. Add 350 µl Buffer VDW1 to the column and centrifuge at 8000×g (or 10000 rpm) for 1 minute. Discard the flow-through.
18. Add 500 µl Buffer VDW2 to the column and centrifuge for 2 min at full speed.
19. Place the column in a new 1.5 ml collection tube. Add 50-100 µl Buffer VTE directly to the column membrane and incubate at room temperature for 5 min. Centrifuge for 1 min at full speed to elute the DNA.

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.