Cell & Tissue DNA Extraction Kit

Catalog Number: EXNA075
Size: 50 Tests

For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

EXREprotein™

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Product Description

The Cell & Tissue DNA Extraction Kit uses a centrifugal adsorption column that can specifically bind DNA and a unique buffer system for rapid extraction of genomic DNA from animal tissues and cells. The optimized Binding Buffer and Proteinase K rapidly lyses cells and inactivates intracellular nucleases, and genomic DNA is selectively adsorbed on the silicon matrix membrane in the centrifuge column. A series of rapid rinse-centrifugation steps remove cell metabolites, proteins, and other impurities. The purified genomic DNA is compatible with Southern-blot and enzyme digestion reactions.

The kit is efficient and simple-to-use. Single samples generate highly pure genomic DNA in under 30 minutes.

Limitations

- For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

Precautions

- Always wear appropriate protective clothing and follow safe laboratory procedures.
- Minimize the length of UV exposure when excising the band from the gel to avoid damage to DNA.

Materials Provided & Storage

Shipped at ambient temperature. Proteinase K shipped on blue ice.
Store the kit at 2-30˚C for up to 24 months**.** Proteinase K in glycerol buffer, store at 2-8˚C for 12 months or -15 to -25˚C for 24 months.

Component	Size	Quantity
Balanced Buffer	5 mL	1 Bottle
Lysate Buffer	11 mL	1 Bottle
Binding Buffer	11 mL	1 Bottle
Inhibitor Removal Solution	25 mL	1 Bottle
Wash Buffer	15 mL	1 Bottle
Elution Buffer	15 mL	1 Bottle
Proteinase K	1 mL	1 Bottle
Adsorption Column	50 Tubes	1 Pack
Collection Tube	50 Tubes	1 Pack

Additional Materials Required, Not Supplied

1X PBS	RNase A
Ispropanol	1.5 mL centrifuge tubes
Ethanol (96-100%)	

Preparation of Reagents

Wash Buffer: Add 60 mL ethanol (96-100%) to the Wash Buffer before use.

Protocol

Column equilibration: The adsorption column should be pretreated with Balanced Buffer before the experiment to improve the nucleic acid binding ability. Place the Adsorption Column into the Collection Tube, add 100 µL of Balanced Buffer to each tube pairing, centrifuged at 13,000×g for 60 seconds, discard the waste liquid from the Collection Tube, and place the Adsorption Column back into the Collection Tube for use later.

Cultured Cell Preparation:

1. Add 10^6-10^7 suspended cells to 1.5 mL centrifuge tube (do not exceed 10^7 cells). For adherent cells, treat with Trypsin.
2. Centrifuge at 13,000 x g for 60 seconds to remove the supernatant.
3. Resuspend cells in 200 µL of 1X PBS, centrifuge at 13,000 x g for 60 seconds, discard the supernatant, and resuspend the cells in 180 µL of 1X PBS.
4. Add 20 µL of Proteinase K solution, mix thoroughly, then add 200 µL Binding Buffer, vortex thoroughly, and place at 70°C for 10 minutes. **Optional:** *if RNA removal is required, add 20 µL RNase A (25 mg/mL), shake to mix, and leave at room temperature for 5-10 minutes after the completion of step 4.*
5. Proceed to Extraction Protocol.

Animal Tissue Preparation:

1. Add 180 µL of Lysate Buffer to fresh or thawed tissue (≤ 25 mg), homogenize on ice.
2. Add 20 µL of Proteinase K solution and vortex thoroughly.
3. Place the lysates in a water bath at 56°C for 1-3 hours or until tissue digestion is complete, shaking gently several times to assist lysis. **Optional:** *if RNA removal is required, add 20 µL RNase A (25 mg/mL), shake to mix, and leave at room temperature for 5-10 minutes after the completion of step 3.*
4. Add 200 µL Binding Buffer, vortex thoroughly, and place at 70°C for 10 minutes.
5. Proceed to Extraction Protocol.

Extraction Protocol:

1. Allow sample to cool to room temperature. Add 100 µL isopropanol, immediately vortex to mix thoroughly.
2. Place the Adsorption Column into the Collection Tube. Add the mixture (including the flocculent precipitate) to the Adsorption Column, centrifuge at 13,000 x g for 60 seconds, and discard the waste liquid.
3. Add 500 µL of Inhibitor Removal Solution, centrifuge at 12,000 x g for 60 seconds, and discard the waste liquid.
4. Add 600 µL of Wash Buffer (per Preparation of Reagents), centrifuge at 12,000 x g for 60 seconds, discard the waste liquid, and repeat this step once.
5. Place the Adsorption Column back into the Collection Tube and centrifuge at 13,000 x g for 2 minutes to thoroughly remove the Wash Buffer and minimize residual ethanol affecting the downstream reaction.
6. Place the Adsorption Column in a new 1.5 mL centrifuge tube, add 100 µL Elution Buffer to the center of the membrane and place it at room temperature for 3-5 minutes.
7. Elute genomic DNA by centrifugation at 12,000 x g for 60 seconds and store at -20°C. **Note:** *the eluted solution can be pipetted back into the Adsorption Column, allowed to stand for 3-5 minutes, and centrifuged at 12000 x g for 60 seconds to increase the DNA concentration. Preheating the Elution Buffer to 65-70℃ in advance may improve results.*