canvax

DATA SHEET

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1. Identification

Product name — **HigherPurity™ Tissue DNA Purification Kit**

Cat. No. **AN0210 (50 reactions)**
Cat. No. **AN0211 (100 reactions)**
Cat. No. **AN0211-XL (250 reactions)**

2. Description

HigherPurity™ Tissue Genomic DNA Purification Kit offers a rapid and convenient method for purification of total DNA from a variety of tissue. The kit is based in DNA ability to bind silica in the presence of high concentrations of chaotropic salts. Eluted purified DNA is suitable and ready-to-use for PCR, real-time PCR, Southern Blotting and RFLP.

The kit can be adapted to extract genomic DNA from animal cells, blood, bacteria, paraffin fixed tissue, yeast and fungi.

3. Kit Contents

Item	Quantity		
	AN0210 (50 rxn)	AN0211 (100 rxn)	AN0211-XL (250 rxn)
Buffer BLY1	11 ml	22 ml	55 ml
Buffer BLY2	11 ml	22 ml	55 ml
Wash Buffer 1 *(WB1)	18.5 ml	37 ml	100 ml
Wash Buffer 2 * (WB2)	8 ml	16 ml	40 ml
Elution Buffer (EB)	10 ml	20 ml	50 ml
Proteinase K **	11 mg	2 x 11 mg	5x11 mg
DNA mini-spin column	50	100	250
Collection tube (2mL)	100	200	500
1.5 ml microtube	50	100	250
Micropestle	50	100	250

*Add the volume ethanol (96%-100%) specified [Not included] to WB1 and WB2 Buffer prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

**Dissolve Proteinase K in water to obtain a 10 mg/mL stock solution. The Proteinase K solution can be stored for several days at 2–8 °C. For longer-term storage, the unused portion of the solution may be stored in aliquots at –20 °C until needed.

! Buffers provided in this kit contain irritants. Wear gloves and lab coat when handling them.

4. Storage specifications

The kit is shipped at ambient temperature. Upon arrival, store Proteinase K **at 4°C**. All other kit components can be stored at room temperature. The kit components are stable for 2 years, if stored properly.

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5. Features

- **High yields**: up tp 50µg; depends on type of sample.
- **Ready to use** DNA.
- **Just a few minutes procedure (about 60 min).**
- **Mini format**

6. Applications

All molecular biology applications, such as: RT-PCR, Southern blotting, RFLP, etc.

7. Recommended Protocol

Things to do before starting:

- Prepare dry baths or water baths before the operation: one to 60 ˚C for step 3 and the other to 70 ˚C for step 5.
- Preheat the Elution Buffer to 70 ˚C for step 12.

Protocol for Isolation of DNA from Animal Tissue

1. Cut 25 mg of animal tissue and transfer to a 2ml microcentrifuge tube (not provided). *Use the micropestle to grind the material to pulp. You can grind the tissue samplein liquid nitrogen.*
2. Add 200µl of Buffer BLY1 and continue to homogenize the sample by grinding.
3. Add 20µl of Proteinase K (10mg/ml), mix by shaking vigorously, and incubate at 60°C for 60-120 minutes (Until the tissue is lysed completely). During incubation, invert the tube regularly.
4. [Optional step; If RNA-free DNA is required] Allow the mixture to cool to room temperature and add 4µl of RNase A (100mg/ml) [not provided], mix by shaking vigorously and incubate for 3 minutes at room temperature.
5. Add 200µl of Buffer BLY2, mix by shaking vigorously, and incubate at 70°C for at least 10 minutes. During incubation, invert the tube regularly.
   (*Note that sample lysate should become clear. If there is still insoluble material present following the lysis step, centrifuge for 2 minutes at 14000g-16000g and transfer the supernatant to a new 1.5- ml microcentrifuge tube*).

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6. Add 200µl of absolute ethanol [not provided] to the lysate and mix immediately by shaking vigorously for 10 seconds. In case precipitate appears, break it up by pipetting.
7. Place the DNA mini-spin column in a 2 ml collection tube and transfer the sample mixture (including any precipitate if present) to the column.
8. Centrifuge at 14,000g-16,000g for 2 minutes. Discard the collection tube containing the flow-through and place the DNA mini spin column in a new collection tube.
9. Add 400µl of Buffer WB1 and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through and place the DNA mini spin column back in the collection tube.
10. Add 750µl of Buffer WB2 and centrifuge at 14,000g-16,000g for 1 minute.
11. Discard the flow-through and place the DNA mini spin column back in the collection tube and centrifuge for another 3 minutes at 14,000g-16,000g to dry the matrix ofthe column.
12. Transfer the DNA spin column to a new 1.5 ml microcentrifuge tube and pipet 100µl preheated Elution Buffer directly to the centre of the spin column without touching the membrane. Incubate at room temperature 5 minutes.

    Notes: Instead of Elution Buffer, DNA can also be eluted with TE or water; pH should be 8.0-8.5. Standard elution volume is 100µl. To increase concentration, elute with 30-50µl. To increase yield, elute with 200µl.
13. Centrifuge for 1 minute at 14,000g-16,000g to elute purified genomic DNA. Discardthe DNA spin column and use DNA immediately or store at -20°C.

Protocol for Isolation of DNA from Animal Cultured Cells

Additional Requirements:

- RNase A (optional)
- 96–100% ethanol
- Trypsin or a cell scraper (for monolayer cells)
- PBS

1. Harvesting Cells

   a. *For cells grown in suspension:*

   i. Transfer the appropriate number of cells (up to 1×10^7) into a microcentrifuge tube.

   ii. Centrifuge at 300 x g for 5 minutes. Carefully and completely discard the supernatant.

   b. *For cells grown as a monolayer:*

   i. Detach cells from the dish or flask using trypsinization or a cell scraper, then transfer the appropriate number (up to 1×10^7) into a microcentrifuge tube.

   ii. Centrifuge at 300 x g for 5 minutes. Carefully and completely discard the supernatant.

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2. Resuspend the cell pellet in PBS to a final volume of 200 µl.

3. Follow the Animal Tissue Protocol starting from step 2.

Protocol for Isolation of Genomic DNA and Viral DNA from Blood

Additional Requirements:

- *RNase A (optional)*
- *96–100% ethanol*
- *PBS*

1. Transfer up to 200 µl of your sample (whole blood, serum, plasma, body fluids, or buffy coat) into a microcentrifuge tube. If the volume is less than 200 µl, add the necessary amount of PBS to bring it to 200 µl.

2. (Optional) If RNA free of genomic DNA is desired, add 4 µl of RNase A at 100 mg/ml (not provided). Vortex the tube thoroughly and let it stand at room temperature for 2 minutes.

3. Add 20 µl of Proteinase K to the sample, then introduce 200 µl of Buffer BLY2. Mix the contents by pulse-vortexing, and incubate at 60°C for 30 minutes. Vortex the tube occasionally during incubation.

4. Following that, incubate the sample at 70°C for 10 minutes.
5. Finally, proceed with the Animal Tissue Protocol starting from step 7..

Protocol for Isolation of DNA from Bacteria

Additional Requirements:

- *RNase A (optional)*
- *96–100% ethanol*
- *For Gram-positive bacteria: a lysozyme reaction solution is recommended (20 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton)*

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I. For Bacterial Cultures

1. Transfer 1 ml of a well-grown bacterial culture into a microcentrifuge tube (not provided).
2. Pellet the cells by centrifuging at full speed (approximately 18,000 x g) for 2 minutes and completely discard the supernatant.
3. Follow the Animal Tissue Protocol starting from step 2.

II. For Bacteria in Biological Fluids

1. Collect the cells by centrifuging the biological fluid at 7,500 rpm (approximately 5,000 x g) for 10 minutes; discard the supernatant completely.
2. Follow the Animal Tissue Protocol starting from step 2.

III. For Bacteria from Eye, Nasal, Pharyngeal, or Other Swabs

1. Soak the swabs in 2 ml of PBS at room temperature for 2–3 hours.
2. Pellet the cells by centrifuging at 7,500 rpm (approximately 5,000 x g) for 10 minutes and completely discard the supernatant.
3. Follow the Animal Tissue Protocol starting from step 2.

IV. For Gram-Positive Bacteria

1. Transfer 1 ml of a well-grown bacterial culture into a microcentrifuge tube (not provided).
2. Pellet the cells by centrifuging at full speed for 2 minutes and completely discard the supernatant.
3. Resuspend the cell pellet in 200 µl of the lysozyme reaction solution (20 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton) and incubate at 37°C for 30–60 minutes.
4. (Optional) If RNA free of genomic DNA is required, add 4 µl of RNase A (100 mg/ml, not provided), mix thoroughly by vortexing, and incubate at room temperature for 2 minutes.
5. Add 20 µl of Proteinase K to the sample, then add 200 µl of Buffer BLY2. Mix thoroughly by pulse-vortexing and incubate at 60°C for 30 minutes, vortexing occasionally during incubation.
6. Further incubate the sample at 95°C for 15 minutes.
7. Follow the Animal Tissue Protocol starting from step 7.

Protocol for Isolation of DNA from Yeast and Fungi

Additional Requirements:

- *RNase A (optional)*
- *96–100% ethanol*
- *Zymolyase or lyticase (200 U per preparation)*
- *Sorbitol buffer (1 M sorbitol; 100 mM EDTA; 14 mM ß-mercaptoethanol)*

Note: Depending on the fungal species and cell wall composition, incubation time and/or enzyme concentration may require optimization.

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1. Transfer 3 ml of log-phase yeast culture (OD600 = 1) into a microcentrifuge tube (not provided). For fungal samples (multicellular fungi) use up to 150 mg of fungal material per extraction.

   Note: For samples that are difficult to homogenize (e.g. fruiting bodies), pre-grind the material to a fine powder under liquid nitrogen before proceeding.

2. Pellet the cells by centrifuging at 7,500 rpm (approximately 5,000 x g) for 10 minutes, then completely discard the supernatant.

3. Resuspend the cell pellet in 600 µl of the sorbitol buffer. Add 200 U of zymolyase or lyticase, and incubate at 30°C for 30 minutes.

4. Centrifuge at 7,500 rpm (approximately 5,000 x g) for 5 minutes, and remove the supernatant.
5. Proceed with the Animal Tissue Protocol starting from step 2.

   Note: If column clogging is observed during the DNA binding step, centrifuge the lysate for 2 minutes after the Proteinase K incubation and transfer only the clear supernatant to continue with the protocol. This additional clarification step helps to prevent column obstruction

Protocol for Isolation of DNA from Fixed Tissue

Additional Equipment:

- *RNase A (optional)*
- *96–100% ethanol*
- *Xylene*

I. For Paraffin-Embedded Tissues

1. Transfer up to 25 mg of paraffin-embedded tissue into a microcentrifuge tube (tube not provided).
2. Add 1 ml of xylene, mix thoroughly, and incubate at room temperature for 30 minutes.
3. Centrifuge at full speed for 5 minutes, then remove the supernatant completely.
4. Add 1 ml of ethanol (96–100%) to the deparaffinized tissue and gently mix by vortexing.
5. Centrifuge at full speed for 3 minutes, and carefully remove the supernatant by pipetting.
6. Repeat steps 4 and 5.
7. Incubate the sample at 37°C for 10–15 minutes to ensure complete evaporation of any ethanol residue.
8. Grind the tissue using a micropestle or liquid nitrogen, then proceed with the Animal Tissue Protocol

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starting from step 2.

II. For Formalin-Fixed Tissues

1. Wash a 25 mg tissue sample twice with 1 ml of PBS to remove formalin.
2. Grind the tissue using a micropestle or liquid nitrogen, then continue with the Animal Tissue Protocol starting from step 2.

8. Further information

Product Use Limitations

This product is developed, designed, and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.canvaxbio.com where you can find, view, and print the MSDS for each CANVAX kit.