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DATA SHEET

Version: 03
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1. Identification

Product name **HigherPurity™ Blood & Cell Culture DNA Extraction Mini Spin Kit**

Cat. No **AN0044 (50 reactions)**
Cat. No **AN0045 (100 reactions)**
Cat. No **AN0045-XL (250 reactions)**

2. Description

HigherPurity™ Blood & Cell culture DNA Extraction Mini Spin Kit is a simple and rapid method for high-quality genomic DNA purification from various sources, including: whole blood, plasma, serum, buffy coat and cell culture.
The kit is based in DNA ability to bind silica in the presence of high concentrations of chaotropic salts.

3. Kit Components

Item	Quantity		
	AN0044 (50 rxn)	AN0045 (100 rxn)	AN0045-XL (250 rxn)
Minispin columns	50	100	250
Collection tubes (2 mL)	100	200	500
BLU Buffer	20 ml	35 ml	75 ml
Proteinase K*	30 mg	2 x 30 mg	5 x 30 mg
WB1 Buffer**	18.5 ml	37 ml	80 ml
WB2 Buffer**	20 ml	40 ml	100 ml
EB Buffer	12 ml	24 ml	60 ml

*Dissolve Proteinase K in water to obtain a 20 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. This product as supplied is stable at room temperature.

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

4. Kit Storage

Store the kit at room temperature. If any kit reagent forms a precipitate, warm at 55–65 °C until the precipitated dissolves and allow to cool to room temperature before use.

5. Features

- ✓ **Safe:** no phenol-chloroform extraction.
- ✓ **Efficient:** 3-6 µg of genomic DNA from a 200 µl blood sample or 15–20 µg from Cultured Cells (5 x 10^6).
- ✓ **Ready to use** genomic DNA, for all molecular biology applications.

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6. Applications

gDNA purified with the kit is suitable for direct use in all common molecular biology applications: PCR, cloning, DNA sequencing, Southern blot analysis, etc.

7. Quality Certifications

HigherPurity™ Blood & Cell culture DNA Extraction Mini Spin Kit is tested on a lot-to-lot basis by isolating total DNA from 250 µl of whole human blood. DNA purified is analysed by:

- ✓ Spectrophotometer: Ratio 260/ 280 (1.6-1.8)
- ✓ Agarose gel electrophoresis.

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.

PROTOCOL A: DNA Purification from Blood (250 µL)

This protocol is for purification of total DNA from whole blood, plasma, serum or buffy coat.

1. Transfer **25 µL** proteinase K into the bottom of a 1.5 ml microcentrifuge tube (not provided).

2. Add **250 µL** of **sample.**

Use up to 250 µl whole blood, plasma, serum or buffy coat. If the sample volume is less than 250 µl, add the appropriate volume of PBS.

For samples larger than 250 µl, the amount of lysis buffer, proteinase K and ethanol used should be increased proportionally, while the volumes of wash and elution buffers should remain constant. For example, 400 µl sample will require 40 µl Protease K, 400 µl Buffer BLU and 400 µl Ethanol.

Buffer BLU and Proteinase K can be purchased separately to supplement the Kit.

3. [Optional Step] RNA Degradation:

If RNA-free gDNA is required, add **4 µl** of **RNase A (100 mg/ml)** [not provided].

4. Add **250 µL** of **buffer BLU** and mix by vortexing (it is important to observe a homogeneous solution).

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5. Incubate in a water bath at 55 ˚C for 15 minutes.

6. Add **250 µl** of **ethanol** (96–100%) [not provided] and mix by vortexing vigorously.

7. Place the **minispin column** in a collection tube and transfer the mix by pipetting. Centrifuge at 8000rpm for 1 minute. Discard the flow-through solution.

8. Place the minispin column in the same collection tube and add **500 µL** of **WB1 buffer**. Centrifuge at 8000 rpm for 1 minute. Discard the flow-through solution.

9. Place the minispin column in the same collection tube and add **500 µL** of **WB2 buffer**. Centrifuge at 8000 rpm for 1 minutes. Discard the flow-through solution.

10. Place the minispin column in the same collection tube and add **800 µL** of **WB2 buffer**. Centrifuge at 8000 rpm for 1 minutes. Discard the flow-through solution.

11. Centrifuge at full speed for 3 minutes to dry the minispin column.

This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

12. Place the minispin column into a new, labelled, 1.5 microcentrifuge tube (not provided) and pipet **100-200 µL EB Buffer** or **pre-warm water** directly into the membrane. Close the tube and incubate for 2 minutes at room temperature.

13. Centrifuge at full speed for 1 minute to elute the DNA

14. The purified genomic DNA can be stored at 2-8˚C for a few days. For longer term storage, -20˚C is recommended.

PROTOCOL B: DNA Purification from Cultured Cells (5x10^6 cells)

1. Transfer the appropriate number of cells (5 x 10^6 cells) to a 1.5 ml microcentrifuge tube.

2. Centrifuge for 5 minutes at 3000 rpm to pellet the cells.

3. Discard the supernatant.

4. Resuspend cell pellet in **PBS** to a final volume of **250 µl.**

5. Add **25 µl proteinase K**

6. Continue with step 3 of **"Protocol A: DNA Purification from Blood"**