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DATA SHEET

Version: 03
Revision date: 21/04/2023

1. Identification

Product name **HigherPurity™ Circulating DNA Purification Kit**

Cat. No **AN0260 (50 reactions)**
Cat. No **AN0260-XL (250 reactions)**

2. Description

HigherPurity™ Circulating DNA Purification Mini Spin Kit is designed for the rapid simultaneous purification of circulating DNA from cell–free samples such as serum and plasma (1000 µl).
The cell-free DNA in plasma /serum is known to be highly fragmented 50-1000 bp. The degree of fragmentation depends on several parameters like origin DNA (fetal, tumor, microbial DNA), health of the donor blood, blood sampling procedure and handling of the sample.
The kit is based in nucleic acid ability to bind silica in the presence of high concentrations of chaotropic salts. For this, we use a special columns designed for high recovery, especially of fragmented DNA in a range 100- 1000 bp.

3. Kit Components

Item	Quantity	
	AN0260 (50 rxn)	AN0260-XL (250 rxn)
Minispin columns	50	250
Collection tubes (2 mL)	250	1250
3 ml tubes	50	250
BLY Buffer	55 ml	275 ml
Proteinase K*	2 x 100 mg	10 x 100 mg
WB1 Buffer**	18 ml	90 ml
WB2 Buffer**	10 ml	50 ml
EB Buffer	2 ml	10 ml

*Dissolve each vial of Proteinase K (100mg) in 1.9 ml of RNase free water to obtain a stock solution.
**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 precipitate dissolves and allow to cool to room temperature before use.
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.

5. Features

- ✓ For rapid isolation of circulating DNA from plasma/serum.
- ✓ Sample size: 1000 µl (plasma or serum).
- ✓ Complete removal of inhibitors.
- ✓ Typical yield: Varies, due to donor-to-donor variations, health status and the level of nucleases present in the blood.
- ✓ Elution volume: 30 µl.
- ✓ DNA obtained that can be directly used in PCR or real-time PCR.

6. Applications

- ✓ The purified circulating DNA is compatible with most of the molecular biological applications.
- ✓ Ideal for detection of biomarkers in various diseases.
- ✓ Analysis of fetal DNA from maternal plasma.

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7. 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.

Samples: Plasma and serum

This kit is suitable for the isolation of DNA from fresh or frozen serum or plasma prepared from blood collected EDTA or citrate (Plasma samples prepared from blood collected on heparin should not be used as heparin can significantly interfere with many downstream applications such as RT-PCR).
Frozen plasma or serum samples should be centrifuged for 2 minutes at 2000 rpm before processing. Only clear supernatant should be processed, as column clogging may be encountered if frozen samples are directly processed.

PROTOCOL

1. Place 1000 µL of sample (plasma or serum) in a 1.5 mL microcentrifuge tube. Incubate at 55°C for 15 minutes. Centrifuge at maximum speed for 5 minutes.
2. Recovery supernatant containing circulating cell-free DNA using a micropipette, avoid touching the visible pellet and add into 3 ml tube supplied. Add **1000 µL of buffer BLY + 75µL Proteinase K** and mix well by vortexing for 10-15 seconds.
3. Incubate at 55 °C for 15 minutes. Mix by inverting or shaking every 5 minutes.
4. **Add 750 µl** Isopropanol and mix with micropipette.
5. Insert the Minispin column into a 2 mL Collection Tube (provided).
6. Transfer 700 µl sample to the Minispin column by pipetting and centrifuge at 8.000rpm for 30 seconds. **The maximum capacity of the minispin columns is 800 uL!,** you will need to repeat this step 4 times on the same column (In the last step centrifuge at 14.000 rpm).
7. Place the Minispin column in a new collection tube and add **500 µL of buffer WB1**. Centrifuge at 14.000 rpm for 1 minute. Discard the flow-through.
8. Place the Minispin column in a new collection tube and add **500 µL of buffer WB2**. Centrifuge at 14.000 rpm for 1 minute. Discard the flow-through.
9. Centrifuge at full speed for an additional 3 min to dry the spin column.
10. Place the Minispin column into a new, labelled 1.5 microcentrifuge tube and pipet **30 µL Elution Buffer (preheated at 70°C)** directly into the membrane. Close the cap and incubate for 2 minutes at room temperature. **It is very important to add the elution buffer in the center of the membrane to be completely wet**.
11. **(Optional)** Centrifuge at 10.000 rpm for 1 minute to elute. Collect the eluate and redeposit in the center of the membrane. Incubate 2 minutes at room temperature. **This optional step increases yield**.
12. Centrifuge at maximum speed for 1 minute. The eluate contains the circulating DNA.
13. For long time storage place the nucleic acids at –20°C.