Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

DATA SHEET

Version: 03
Revision date: 22/03/2023

1. Identification

Product name **HigherPurity™ Stool DNA Isolation Kit**

50 rxn

Cat. No. AN0130

2. Description

HigherPurity™ Stool DNA Isolation Kit provides a simple and convenient technique to isolate high quality DNA from fresh or frozen stool samples. Extraction is based in DNA ability to bind silica in the presence of high concentrations of chaotropic salts as guanidinium thiocyanate. Fecal samples are rapidly and efficiently lysed by bead beating. The sample DNA is then bound to the surface of a Silica Membrane Mini Spin Column and washed, and the bound DNA is then desorbed from the surface of the Spin column. The inhibitors of the downstream PCR will be removed by utilizing the DNA binding column and the buffers system in this kit.

3. Kit Contents

Component	Quantity
Lysis Solution 1 (LS1)	20 ml
Buffer A	10 ml
Inhibitor Removal Buffer (IR-Buffer)	15 ml
Buffer B	15 ml
Wash Buffer 1 * (WB1)	16 ml
EB Buffer	15 ml
Proteinase K**	11 mg
CleanEasy™ MiniSpin Columns	50
Collection tube (2mL)	100
Bead Tube (with glass beads)	50
1.5 ml microcentrifuge tube	50

*Add the volume ethanol (96%-100%) specified [Not included] to WB1 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.

! Caution: Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water.

4. Storage specifications

Store Proteinase K **at 4°C.** All other kit components can be stored at room temperature.

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5. Applications

All molecular biology applications,such as:

- Digestion with restriction enzymes.
- Automated sequencing.
- PCR template.
- Southern Blots.

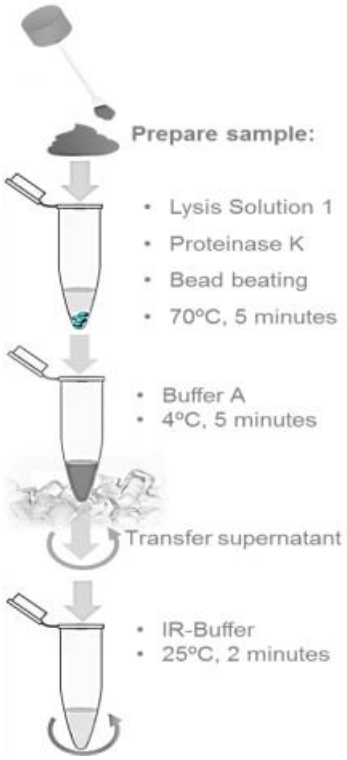
6. Quality Certifications

Soil DNA Isolation Kit is tested for isolation of DNA from soil sample. The quantity and quality of purified DNA attend to:

- Ratio 260/ 280.
- Agarose gel electrophoresis.
- Digestion with restriction endonucleases

7. Assay Procedure

1. Transfer 100-200 mg (or 200 µl for liquid sample) of stool sample into Bead Tube (with glass beads) and place on ice. For stool samples that have been preserved using Canvax's Stool Sample Collection & Stabilization Kit, see the note at the end of protocol.
2. Add **300µl of Lysis Solution 1 (LS1)** and **20 µl of proteinase K (10 mg/ml)**. Vortex for 5 minutes at maximum speed. *Make sure that stool sample is homogenized completely.*
3. Incubate the sample at 70 ˚C for 5 minutes. *Vortex the sample twice during the incubation. For detection of human DNA, it is sufficient to incubate at 70°C. If necessary, the temperature can be increased to 95˚C for isolation of DNA from bacteria or parasites.*
4. Cool down the sample and add **100 µl of Buffer A** to the sample, mix well by vortexing. Incubate the sample on ice for 5 minutes.
5. Centrifuge at full speed for 5 minutes. Pipet the supernatant into a new 1.5 ml microcentrifuge tube (not provided) and discard the pellet. *Avoid pipetting any debris and pellet.*



6. Add **200 µl of IR-Buffer** to the sample, mix well by vortexing. Incubate the sample at room temperature for 2 minutes. *IR-Buffer must be suspended completely by vigorously vortexing before every using.*
7. Centrifuge at full speed for 2 minutes. Carefully pipet 250µl of supernatant into a new 1.5 ml microcentrifuge tube (not provided) and discard the pellet. *Avoid pipetting any debris and pellet.*

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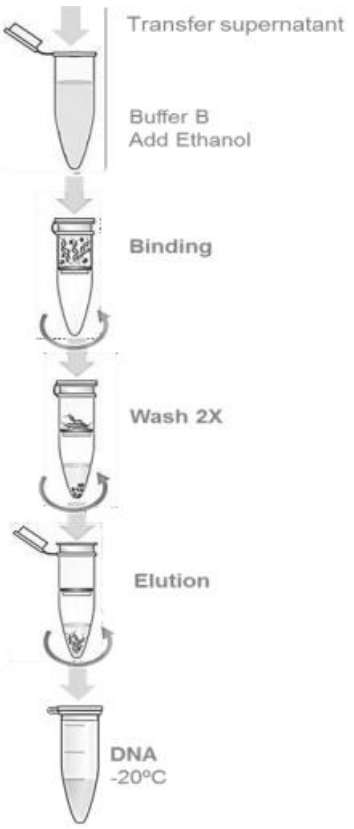
8. Add **250µl of Buffer B** and 250 µl of ethanol (96%~100%). Mix thoroughly by vortexing.
9. Assemble a spin column with one of the provided collection tubes. Apply all the sample mixture onto the spin column. Close the cap and centrifuge at full speed for 1 min. Discard the flow-through and reassemble the spin column to a new Collection Tube.
10. Carefully open the spin column and add **750 µl Buffer WB1** (ethanol added). Close the cap and centrifuge at full speed for 1 min, then discard the flow-through. Return the spin column back to the Collection Tube. **Repeat this step for one more time.**
11. Centrifuge at full speed for an additional 3 min to dry the spin column. *This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.*
12. Place the spin column into a new 1.5 mL microcentrifuge tube. Carefully open the spin column and Add **50~200 µl of Elution Buffer** or ddH2O to the membrane center. Close the cap and incubate for 1 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.
13. The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, - 20°C is recommended.



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.

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Note:

1. For stool samples that have been preserved using Canvax's Stool Sample Collection & Stabilization Kit, add 1 mL of preserved sample to a provided Bead Tube and mix well by vigorously vortexing for 1 minute.
   Before transferring the sample, make sure that the sample is completely homogenized.
2. For detection of human DNA, incubate 10 min at room temperature under shaking.
   For enrichment of Bacterial DNA, Incubate the sample at 70 ˚C for 20 minutes. Vortex the sample twice during the incubation. The lysis temperature can be increased to 95˚C for cells that are difficult to lyse (such as Gram-positive bacteria).
3. Centrifuge at full speed for 5 minutes. Carefully pipet 600µl of supernatant into a new 2 ml microcentrifuge tube (not provided) and discard the pellet.
   Avoid pipetting any debris and pellet: A pellet will appear and in the surface a layer of fat, to introduce the pipette tip crossing this superficial layer of fat, only trying to pick up 600 µl of supernatant that it is the transparent liquid with color.
4. Add 250 µl of IR-Buffer to the sample, mix well by vortexing. Incubate the sample at room temperature for 5 minutes. IR-Buffer must be suspended completely by vigorously vortexing before every using.
5. Centrifuge at full speed for 2 minutes. Carefully pipet 400µl of supernatant into a new 1.5 ml microcentrifuge tube (not provided) and discard the pellet. Avoid pipetting any debris and pellet
6. Add 25 µl of proteinase K (30 mg/ml). Mix thoroughly by vortexing.
7. Incubate the sample at 70 ˚C for 10 minutes.
8. Add 400µl of Buffer B and 400 µl of ethanol (96%~100%). Mix thoroughly by vortexing.
9. Transfer the sample mixture in two steps: Assemble a spin column with one of the provided collection tubes. Apply half of the sample mixture onto the spin column. Close the cap and centrifuge at full speed for 1 min. Discard the flow-through and reassemble the spin column to the same Collection Tube. Repeat the step again with the other half.
10. Follow the previous Protocol starting from step 10.