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Stool DNA Purification Kit

06/20

(Catalog # K1474-50, -250; 50 or 250 Preps; Store at Multiple Temperatures)

I. Introduction:

The Stool DNA Purification Kit is designed for the rapid and reliable purification of high-quality DNA from stool or fecal samples. Up to 0.25 gm of stool samples can be processed in less than an hour. The kit protocol does not need organic extraction and multiple samples can be processed in parallel. The kit combines the reversible nucleic acid-binding properties of spin columns with a propriety buffer system to eliminate PCR inhibiting compounds such as humic acid from stool samples. In this kit, stool samples are homogenized and then treated in a specially formulated buffer that contains a detergent. Humic acid, proteins, polysaccharides and other contaminants are subsequently precipitated after a heat-freeze step. DNA is further purified using a spin-column. A rapid wash step removes trace contaminants and pure DNA is eluted in sterile water or low ionic strength Elution Buffer. Purified DNA can be used in downstream applications such as PCR, restriction digestion, and hybridization techniques.

II. Application:

- To extract DNA from stool or fecal samples

III. Key Features

- Rapid, easy and convenient
- **Highly pure**, high yield
- **Many downstream applications** such as PCR, restriction digestion, and hybridization techniques
- High quality spin columns

IV. Sample Types:

- Stool or fecal samples

V. Kit Contents:

Components	K1474-50 (50 Preps)	K1474-250 (250 Preps)	Part Number
Dry Beads Tube	50	250	K1474-XX-1
Beads solution	42 ml	210 ml	K1474-XX-2
Buffer FLX	40 ml	200 ml	K1474-XX-3
Buffer P2	15 ml	75 ml	K1474-XX-4
DH Reagent	12 ml	60 ml	K1474-XX-5
Buffer BL	30 ml	150 ml	K1474-XX-6
DNA Columns	50	250	K1474-XX-7
DNA Wash Buffer	15 ml	2 x 24 ml	K1474-XX-8
Elution Buffer	20 ml	100 ml	K1474-XX-9
2 ml microcentrifuge tubes	100	500	K1474-XX-10
RNase A	160 µl	800 µl	K1474-XX-11

DNA Wash Buffer must be diluted with 100% ethanol before starting. Add 60 ml (K1474-50) or 96 ml (K1474-250) to DNA Wash Buffer bottle before use. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.

VI. User Supplied Reagents and Equipment:

- Pipettes, Pipette tips
- 100% Ethanol, Sterile water
- Isopropanol
- Sterile, nuclease-free 1.5 ml or 2 ml microcentrifuge tubes
- Microcentrifuge
- Waterbath

VII. Shipping and Storage Conditions:

The kit is shipped and stored at Room Temperature (RT). RNase A must be stored at 4°C. The kit reagents will be stable for 12 months if stored properly.

VIII. Reagent Preparation and Storage Conditions:

1. DNA Wash Buffer must be diluted with 100% ethanol before starting. Add 60 ml (K1474-50) or 96 ml (K1474-250) to DNA Wash Buffer bottle before use.
2. Preheat Buffer FLX and Elution Buffer at 65°C. Make sure the crystals in Buffer FLX are completely dissolved.
3. Buffer BL contains chaotropic salts, which may form reactive compounds with bleach. Do not add bleach or acidic solutions directly to the preparation waste. Wear gloves and protective eyewear when handling this buffer.
4. Precipitates may form in some buffers. Incubate the solutions at 65°C.

IX. DNA Purification:

1. Add **0.25 gm stool sample** to a **Dry Beads Tube**. Add 700 µl of **Beads solution**. Vortex briefly for 5 sec. Add 70 µL **Buffer FLX**. Vortex at maximum speed for 5 min or until the sample is thoroughly homogenized.
2. Incubate at 70°C for 10 min. Mix samples twice during incubation by vortexing the tube.
   Optional: For isolation of DNA from gram positive bacteria, perform a second incubation at 95°C for 2 min. Spin the sample at 10,000 x g for 1 min. Transfer 500 µl of the clear lysate to a 2 ml tube.
   Make sure the stool vial rotates freely in your centrifuge without rubbing. Centrifuge the tubes at 10,000 x g for 30 sec at RT. CAUTION: Be sure not to exceed 10,000 x g.

3. Add 250 µl **Buffer P2**. Mix thoroughly by vortexing for 30 sec. Incubate the samples on ice for 5 min.
4. Centrifuge the sample at 10,000 x g for 2 min. Carefully transfer about 600 µl of supernatant, avoiding the pellet, to a 1.5 ml microcentrifuge tube.
5. Add 2-3 µl of **RNase A** to 250 µL of **Buffer DH** and add to supernatant. Mix well by vortexing for 5 sec and incubate on ice for 5 min.
6. Centrifuge at 10,000 x g for 2 min at RT.
7. Transfer about 700 µl supernatant, avoiding the pellet to a clean 2 ml microcentrifuge tube, and add 1 ml of **Buffer BL** and 100 µl **isopropanol**. Mix well by vortexing for 5 sec.
8. Transfer 700 µl of the sample to a **DNA column** and spin at 10,000 x g for 30 sec. Discard the flow-through and re-use the microcentrifuge tube. Process the remaining sample similarly.
9. Add 700 µl of **DNA Wash Buffer** to the DNA column and spin at 10,000 x g for 30 sec. Discard the flow-through and put the column back in the microcentrifuge tube.
10. Centrifuge the empty column at maximum speed for 2 min. Transfer the column to a 1.5 ml microcentrifuge tube.
11. Add 50 µl of **Elution Buffer** directly to the center of the DNA column and incubate at 65°C for 5 min.
12. Centrifuge at 10,000 x g for 1 min to **elute** the DNA.
13. Optional: Re-apply the eluted DNA to the column and spin at 10,000 x g for 1 min for a second elution to increase the yield.

X. General Troubleshooting Guide:

Problem	Possible Reason	Suggested Improvement
$A_{260/230}$ ratio is low	Inefficient elimination of inhibitory compounds	Repeat the DNA isolation with a new sample, be sure to mix the sample with DH reagent thoroughly and extract the sample with DH reagent twice.
	Salt contamination	1. Repeat the DNA isolation with a new sample. 2. Make sure the column is dried before elution. 3. Wash the column with extra DNA Wash Buffer.
	DNA Wash Buffer prepared with lower percentage ethanol	Prepare DNA Wash Buffer with 100% ethanol.
A260/280 ratio is high	High RNA contamination	Be sure to treat the sample with RNase A. See step 5.
Low DNA yield or no DNA eluted	Samples stored incorrectly	Sample should be stored at -20°C.
	Poor homogenization of sample	Repeat the DNA isolation with a new sample. Be sure to mix the sample with Buffer FLX and glass beads. Increase the time for beads beating to make sure the samples are fully homogenized and cells are lysed.
	Incorrect Buffer BL was added before loading to the column	Repeat the DNA isolation with a new sample.
	DNA was lost in washing	Dilute DNA Wash Buffer Concentrate by adding appropriate volume of 100% ethanol prior to use.
Problems in downstream applications	Ethanol residue present in the eluate	Be sure to completely dry the column before elution.
Little or no supernatant after initial centrifugation step	Insufficient centrifugal force	Check the centrifugal force and increase the centrifugation time if necessary.

XI. Related Products:

BioVision Product Name	Cat. No.	Sizes
ExoDNAPS™ Circulating and Exosome-associated DNA Extraction Kit (Human Plasma/Serum, 20 reactions)	K1230-20, -40	20, 40 Reactions
ExoDNAUC™ Circulating and Exosome-associated DNA Extraction Kit (Urine/Cell Media, 20 reactions)	K1231-40	40 Reactions
Soil Genomic DNA Kit	K1411-50, -250	50, 250 Preps
Yeast Genomic DNA Kit	K1414-50, -250	50, 250 Preps
Fungal Genomic DNA Kit	K1415-50, -250	50, 250 Preps
Buccal Swab DNA Purification Kit	K1466-50, -250	50, 250 Preps
Eye, Nose & Swabs gDNA Purification Kit	K1467-50, -250	50, 250 Preps
Saliva DNA Purification Kit	K1468-50, -250	50, 250 Preps
Bacterial Genomic DNA Isolation Kit	K309	100 Preps
Bacterial DNA Purification Kit II	K1457-50, -250	50, 250 Preps

FOR RESEARCH USE ONLY! Not to be used on humans.