

Fecal DNA Purification Kit

(Spin Columns)

Qualitative Assay for
Manual Extraction Systems

Instructions For Use



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Version 1. GE-011.03.23



GE-011

GE_011/50 – Fecal DNA Purification Kit – 50 rxn

GE_011/250 – Fecal DNA Purification Kit – 250 rxn

GE_011/500 – Fecal DNA Purification Kit – 500 rxn



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EC **REP**

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Description of the Kit Components, Transportation and Storage

Table 1. Content of the Kit

Fecal DNA Purification Kit		50 rxn	250 rxn	500 rxn	Transportation and Storage
Solution A1	Lysis Buffer	26 ml	65 ml x 2	85 ml x 3	Room temperature
Solution A2	Neutralization Buffer	6 ml	26 ml	51 ml	Room temperature
Solution A3	Synthetic Activator	6 ml	26 ml	51 ml	Room temperature
Solution B	Binding Buffer	16 ml	80 ml	80 ml x 2	Room temperature
Solution W1 (conc.)	Wash Buffer 1	8 ml	21 ml x 2	28 ml x 3	Room temperature
Solution W2 (conc.)	Wash Buffer 2	5 ml	14 ml x 2	18 ml x 3	Room temperature
Solution E	Elution Buffer	11 ml	51 ml	105 ml	Room temperature
Proteinase K	Enzyme <i>Lyophilized Powder</i>	20 mg	20 mg x 5	20 mg x 10	Room temperature
Proteinase K Storage Buffer	Storage Buffer for Enzyme	1.5 ml	6 ml	15 ml	Room temperature
OxGEn Glass Beads	Solid Glass Beads	50	250	500	Room temperature
G-Spin/Columns	Silica Spin Columns, with Collections	50	250	500	Room temperature
Collection Tubes	Collection Tubes (2 ml)	100	500	1000	Room temperature

Reagents Preparation

Solution W1

Wash Buffer 1 comes as a concentrate. Prior to initial use, combine the recommended quantity of ethanol, which must be at least 95% pure, as specified on the bottle and in Table 2. If the labels of Solution W1 indicate that ethanol has already been added by the manufacturer omit this step.

Table 2. Preparation of Solution Wash 1

No. Reactions	Solution W1	Ethanol $\geq 95\%$	Final Volume
50	5 ml	26 ml	31 ml
250	14 ml x 2	66 ml (In each bottle)	80 ml x 2 bottles
500	18 ml x 3	87 ml (In each bottle)	105 ml x 3 bottles

Solution W2

Wash Buffer 2 comes as a concentrate. Prior to initial use, combine the recommended quantity of ethanol, which must be at least 95% pure, as specified on the bottle and in Table 3. If the labels of Solution W2 indicate that ethanol has already been added by the manufacturer omit this step.

Table 3. Preparation of Solution Wash 2

No. Reactions	Solution W2	Ethanol $\geq 95\%$	Final Volume
50	5 ml	26 ml	31 ml
250	14 ml x 2	66 ml (In each bottle)	80 ml x 2 bottles
500	18 ml x 3	87 ml (In each bottle)	105 ml x 3 bottles

Proteinase K

Proteinase K is supplied as a lyophilized powder. Before using for the first time, add the appropriate amount of Proteinase K Storage Buffer as indicated in Table 4. Aliquoted Proteinase K should be stored at -20 °C.

Table 4. Preparation of Proteinase K Enzyme

No. Reactions	Proteinase K	Proteinase K Storage Buffer	Final Volume
50	20 mg	1 ml	1 ml
250	20 mg x 5	1 ml (In each vial)	1 ml x 5 vials
500	20 mg x 10	1 ml (In each vial)	1 ml x 10 vials

The List of Materials to be Supplied by the User

Table 6. Equipment and Reagents to be Supplied by the User

Equipment	Consumables
Thermoblock or thermomixer	Ethanol \geq 95%
Centrifuge and minifuge	RNase-free 1.5 ml and 2 ml microcentrifuge tubes
Beat Beating Machine or Vortex	0.5 - 10 μ l pipette tips with filter
Pipette 0.5 - 10 μ l	10 – 100 μ l pipette tips with filter
Pipette 20 - 200 μ l	20 – 200 μ l pipette tips with filter
Pipette 100 - 1000 μ l	100 - 1000 μ l pipette tips with filter

Instructions for Manual Purifications

IMPORTANT: The sample should be stored according to “Collecting and Handling of Clinical Specimens for PCR Testing”. The kit removes all traces of humic acid using a combination of chemical and physical homogenization and lysis. However, in some cases preprocessing/dilution of the samples might be needed. Please follow CDC protocol on fecal specimen collection and preservation.

Fecal DNA Purification Kit (Spin Columns) Protocol

Note: Before starting the procedure, prepare the solutions and enzymes according to the solution preparation guide (Table 2-5). Solution A1 may form precipitates upon storage. Warm it up to 60°C until the residues have fully dissolved. **Preheat Solution E at 56°C before starting the procedure.**

1. Weigh 20-60 mg of fecal sample and place in a 2 ml microfuge tube, prefilled with OxGEN Glass Beads;
2. Add 500 µl of Solution A1, 100 µl of Solution A2, and vortex for 15s, then add 100 µl of Solution A3 and vortex for 30s;
3. Add 20 µl of Proteinase K, and mix thoroughly by bead beating machine/vortexing for 1 min. Spin down;
4. Incubate the sample for 15 min at 56 °C in a thermomixer at 1400 rpm. Alternatively, incubate in a thermoblock and vortex periodically at 5 min intervals.
5. Freeze the sample for 10 min at -20°C;
6. Cool down to room temperature (RT), centrifuge at 13 000 rpm for 5 min;
7. Transfer 300 µl of supernatant into a new 2 ml microfuge tube, and add 300 µl of Solution B and 300 µl of 95 % ethanol. Close the cap and gently invert 30 times. Spin down;
Note: A thin layer will be formed on top of the clean supernatant. Carefully collect only the clean supernatant although small pieces of the layer will not affect the quality of the DNA.
8. Transfer 450 µl of lysate into a G-spin/column, and centrifuge at 8 000 rpm for 1 min. Discard the flow-through;
9. Repeat step 8 with the remaining lysate until the entire lysate has passed through the G-spin/column. Change the collection tube;
10. Add 600 µl of Solution W1 and centrifuge at 13,000 rpm for 2 min. Change the collection tube;
11. Add 600 µl of Solution W2 and centrifuge at 13,000 rpm for 2 min. Discard the flow-through;
12. Remove residual buffer by centrifuging at 13 000 rpm for 2 min. Discard the collection tube;
13. Transfer the G-spin/column into a new 1.5 ml microfuge tube;
14. Add 50-150 µl of preheated (56°C) Solution E to the G-spin/column, ensuring the membrane's entire surface is hydrated. Avoid touching the G-spin/column walls with the pipette tip;
15. Incubate for 3 min at room temperature (RT);
16. Elute the DNA by centrifuging at 13,000 rpm for 2 minutes.

Disposal

Dispose of used kit reagents, human clinical samples, and sealed amplification plates as laboratory clinical waste according to local, state, and federal regulations.

Version History

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Quality Control System

Quality management system TÜV SÜD-ISO 9001:2015. Each Fecal DNA Purification Kit batch is tested against predetermined quality specifications to ensure consistent product quality.

Technical support

For technical support, please contact our dedicated Technical Support Team at:
TEL: +995 599 374 374, Email: support@oxgensolutions.com

Explanation of Symbols

LOT Batch code

 Use by

REF Catalogue number

 Store at

QTY Quantity

 Manufactured by