Blood/Tissue DNA Purification Kit

(Spin Columns)

Qualitative Assay for

Manual Extraction Systems

Instructions For Use

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Version 2. GE-006.03.23

REF GE-006

GE_006/50 – Blood/Tissue DNA Purification Kit – 50 rxn

GE_006/250 – Blood/Tissue DNA Purification Kit – 250 rxn

GE_006/500 – Blood/Tissue DNA Purification Kit – 500 rxn

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Description of the Kit Components, Transportation and Storage

Table 1. Content of the Kit

Blood/Tissue DNA Purification Kit		50 rxn	250 rxn	500 rxn	Transportation and Storage
Solution A	Lysis Buffer	11 ml	51 ml	101 ml	Room temperature
Solution B	Lysis/Binding Buffer	11 ml	51 ml	101 ml	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 *Lyophilized Powder*	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
RNase A	Enzyme *Lyophilized Powder*	2 mg	10 mg	10 mg x 2	Room temperature
RNase A Storage Buffer	Storage Buffer for Enzyme	300 µl	1.5 ml	1.5 ml x 2	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 ≥95%	Final Volume
50	8 ml	23 ml	31 ml
250	21 ml x 2	59 ml (In each bottle)	80 ml x 2 bottles
500	28 ml x 3	77 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 ≥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

RNase A

Before using for the first time, add the appropriate amount of RNase A Storage Buffer as indicated in Table 5. Aliquoted RNase A should be stored at -20°C. Do not freeze-thaw more than 5 times.

Table 5. Preparation of RNase A

No. Reactions	RNase A	RNase A Storage Buffer	Final Volume
50	2 mg	0.2 ml	0.2 ml
250	10 mg	1 ml	1 ml
500	10 mg x 2	1 ml (In each vial)	1 ml x 2 vials

Recommended Sample Pretreatment

This kit facilitates the extraction of DNA from blood and tissue samples.

Whole Blood - Before processing, ensure that entire blood samples are fully liquefied to prevent clot carryover, which could interfere with nucleic acid purification.

Plasma/Serum - When handling plasma samples, carefully transfer the clear, yellowish fluid, not disturbing the underlying buffy coat or red blood cell layer.

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 ≥95%
Centrifuge and minifuge	RNase-free 1.5 ml microcentrifuge tubes
Vortex	0.5 - 10 µl pipette tips with filter
Pipette 0.5 - 10 µl	10 – 100 µl pipette tips with filter
Pipette 20 - 200 µl	20 – 200 µl pipette tips with filter
Pipette 100 - 1000 µl	100 - 1000 µl 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”. Before analysis, ensure the samples do not contain inhibiting mucus and sediments.

Blood DNA Purification Protocol

Note: Before starting the procedure, prepare the solutions and enzymes according to the solution preparation guide (Tables 2-5). Solution B 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. Transfer 20 µl of Proteinase K into a 1.5 ml tube;
2. Add 200 µl of the whole blood or plasma/serum sample to the tube and mix by pulse-vortexing for 15s;
3. Add 200 µl of Solution B, mix by pulse-vortexing for 15s and spin down;

Note: If RNA-free genomic DNA is required, add 4 µl of RNase A, mix by vortexing and spin down.

4. Incubate the sample for 15 min at 56˚C in a thermomixer at 1400 rpm. Alternatively, incubate in a thermoblock, vortex and spin down periodically at 5 min intervals;
5. Cool down to room temperature;
6. Add 200 µl of 96% ethanol. Close the cap and gently invert 30 times. Spin down;
7. Carefully transfer 620 µl of lysate into a G-spin/column. Avoid introducing bubbles to the G-spin/column. Centrifuge at 8 000 rpm for 2 min. Change the collection tube;
8. Wash the G-spin/column with 600 µl of Solution W1. During this step, ensure that all traces of lysate are washed off from the walls of the G-spin/column. Centrifuge at 13,000 rpm for 2 min. Change the collection tube;
9. Wash the G-spin/column with 600 µl of Solution W2 and centrifuge at 13,000 rpm for 2 min. Discard the flow-through;
10. Remove residual buffer by centrifuging at 13 000 rpm for 2 min. Discard the collection tube;
11. Transfer the G-spin/column into a new 1.5 ml microfuge tube;
12. Add 50-200 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;
13. Incubate for 3 min at room temperature;
14. Elute the DNA by centrifuging at 13,000 rpm for 2 minutes.

Tissue DNA Purification Protocol

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

1. Cut up to 25-50 mg tissue (up to 10 mg spleen or liver) into small pieces on ice, place in a 1.5 ml microfuge tube or use OxBeads zirconia/steel beads (*not supplied by the manufacturer*). Add 190 µl of Solution A and 20 µl of Proteinase K. Mix thoroughly by bead beating machine or high-speed vortexing for 2-5 min. Spin down the tube to remove drops from inside the lid;
2. Note: If RNA-free genomic DNA is required, add RNase 4 µl, mix by vortexing, and incubate for 2 min at RT.
3. Incubate for 2-24 hours at 56°C in a thermomixer with full-speed agitation (approximately 1400 rpm), or incubate in a thermoblock, vortexing the sample every 30 min;
4. Cool down to room temperature;
5. Note: If the tissue cannot be fully lysed, centrifuge the sample for 2 min at 13 000 rpm and transfer 200 µl of supernatant to a new microcentrifuge tube.
6. Add 200 µl of Solution B and 200 µl of 95% cold ethanol. Close the cap, gently invert 30 times and spin down;
7. Carefully transfer 620 µl of lysate into a G-spin/column. Avoid introducing bubbles to the G-spin/column. Centrifuge at 8 000 rpm for 2 min. Change the collection tube;
8. Wash the G-spin/column with 600 µl of Solution W1. During this step, ensure that all traces of lysate are washed off from the walls of the G-spin/column. Centrifuge at 13,000 rpm for 2 min. Change the collection tube;
9. Wash the G-spin/column with 600 µl of Solution W2 and centrifuge at 13,000 rpm for 2 min. Discard the flow-through;
10. Remove residual buffer by centrifuging at 13 000 rpm for 2 min. Discard the collection tube;
11. Transfer the G-spin/column into a new 1.5 ml microfuge tube;
12. Add 50-200 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;
13. Incubate for 3 min at room temperature;
14. Elute the DNA by centrifuging at 13,000 rpm for 2 min.

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

Instruction for Use Version GE-006.03.23 EN V2, March 10, 2024.

Quality Control System

Quality management system TÜV SÜD-ISO 9001:2015. Each Blood/Tissue 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

Batch code


Use by

Catalogue number


Store at

Quantity


Manufactured by