Pathogen DNA/RNA Purification Kit

(Spin Columns)

Qualitative Assay for

Manual Extraction Systems

Instructions For Use

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Version 3. GE-008.03.24

REF GE-008

GE_008/50 – Pathogen DNA/RNA Purification Kit – 50 rxn

GE_008/250 – Pathogen DNA/RNA Purification Kit – 250 rxn

GE_008/500 – Pathogen DNA/RNA Purification Kit – 500 rxn

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Description of the Kit Components, Transportation and Storage

Table 1. Content of the Kit

Pathogen DNA/RNA Purification Kit		50 rxn	250 rxn	500 rxn	Transportation and Storage
Solution A	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*	5 mg	25 mg	25 mg x 2	Room temperature
Proteinase K Storage Buffer	Storage Buffer for Enzyme	300 µl	1500 µl	1500 µl x 2	Room temperature
G-Spin/Columns	Silica Spin Columns, with Collections	50	250	500	Room temperature
Collection Tubes	Collection Tubes (2ml)	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 bottles)	80 ml x 2 bottles
500	28 ml x 3	77 ml (In each bottles)	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 it 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	5 mg	250 µl	250 µl x 1 vial
250	25 mg	1250 µl	1250 µl x 1 vial
500	25 mg x 2	1250 µl (In each vials)	1250 µl x 2 vials

Recommended Sample Pretreatment

This kit facilitates the extraction of DNA and RNA from viruses, easy-to-lyse bacteria, and parasites.

Urine - Please centrifuge 2 ml urine samples at 2,250 g for 15 minutes before processing. After centrifugation, resuspend the resulting pellet in 200 µl of 1x PBS buffer.

Swabs (Nasopharyngeal, Oropharyngeal, Urogenital) - Swab samples may contain mucus, blood, or other materials that can negatively affect purification. To prepare for processing, thoroughly mix the swab in 1x PBS or 0.9 % saline buffer. Vortex the mixture vigorously at the highest setting for 15 seconds, then transfer the clear sample into a microwell plate.

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 and serum samples, carefully transfer the clear, yellowish fluid, not disturbing the underlying buffy coat or red blood cell layer.

Semen/Cerebrospinal Fluid - Vortex the samples vigorously for no more than 15 seconds, and then transfer the clarified sample into a microwell plate or microcentrifuge tube.

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	RNase-free 1.5 ml microcentrifuge tubes
Vortex	Benchtop cooler or ice box
Pipette 0.5 - 10 µl	0.5 - 10 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.

Pathogen DNA/RNA Purification Kit (Spin Columns) Protocol

Note: Before starting the procedure, prepare the solutions and enzymes according to the solution preparation guide (Table 2-4). Solution A 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 5 µl of Proteinase K into a 1.5 ml tube, and add 200 µl sample. Mix by pulse-vortexing;
2. Pipet 200 µl of Solution A. Mix by pulse-vortexing for 15 sec;
3. Incubate the samples for 15 min at 56 °C in a thermomixer at 1400 rpm. Alternatively, incubate in a thermoblock and vortex periodically at 5 min intervals. Spin down the tube to remove drops from inside the lid;
4. Cool down the sample at Room Temperature (RT). Add 200 µl of 96–100% cold ethanol, invert gently 30 times and spin down.
5. Transfer 600 µl of lysate into a G-spin/column and centrifuge at 8 000 rpm for 2 min. Discard the collection tube;
6. Wash the G-spin/column with 600 µl of Solution W1 and centrifuge at 13 000 rpm for 2 min. Discard the collection tube;
7. Wash the G-spin/column with 600 µl of Solution W2 centrifuge at 13 000 rpm for 2 min. Discard the flow-through;
8. Remove residual buffer by centrifuging at 13 000 rpm for 2 min. Discard the collection tube;
9. Transfer the G-spin/column into a new 1.5 ml microcentrifuge tube;
10. Add 50-200 µl of preheated (56°C) Solution E to the G-spin/column, ensuring the membrane's surface is hydrated. Avoid touching the membrane with the pipette tip;
11. Incubate the samples for 3 min at RT.
12. Elute the DNA/RNA by centrifuging at 13 000 rpm for 1 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

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Quality Control System

Quality management system TÜV SÜD-ISO 9001:2015. Each Pathogen DNA/RNA 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