FECAL AND URINE DNA PURIFICATION KIT

Product Manual

26-017.....5 preps
26-014B.....50 preps
P/N 03-293-8 REV. D

OMNI International
The Homogenizer Company

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INTRODUCTION

The Omni Fecal/Urine DNA Kit allows rapid and reliable isolation of high-quality host genomic DNA, gram positive and negative bacterial DNA, fungal spore DNA, and viral DNA and RNA from urine and fecal samples. Elution volumes as low as 15 µL can be used to maintain higher nucleic acid concentrations.

OVERVIEW

The system combines the Omni's Micro DNA Columns with BBR Buffer to eliminate PCR inhibiting compounds within the samples and elute highly concentrated DNA. Purified DNA is suitable for PCR, restriction digestion, and hybridization applications. There are no organic extractions thus reducing plastic waste and hands-on time and multiple samples can be processed in parallel.

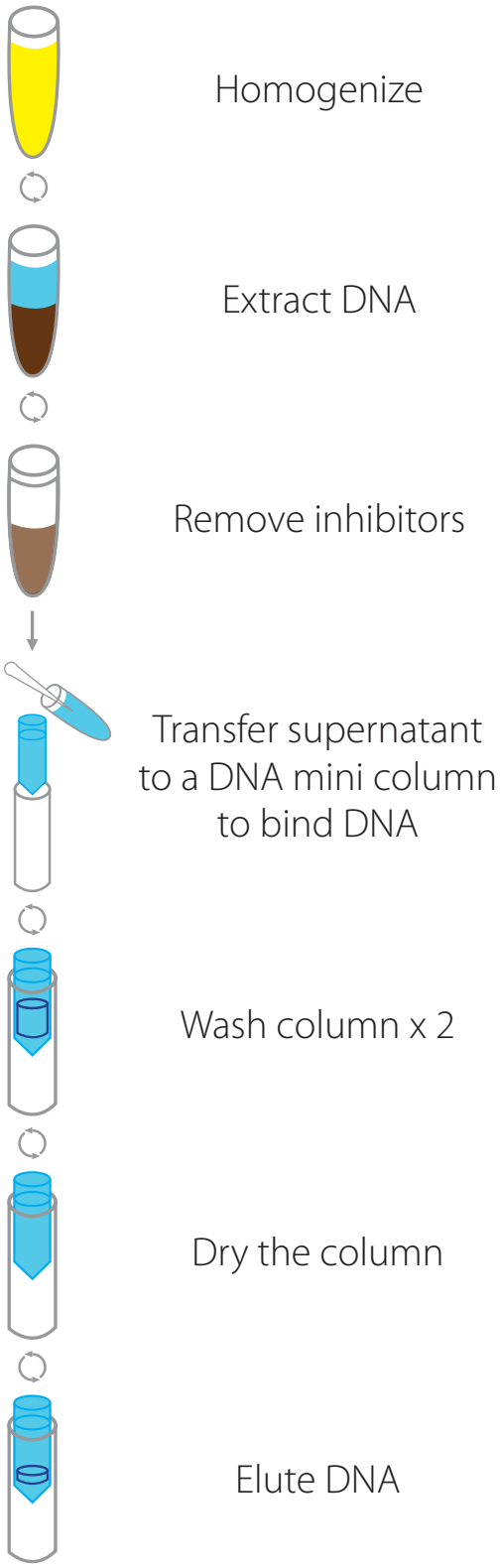
KIT CONTENTS

Product Number	26-017	26-014B
Purifications	5	50
Micro™ DNA Columns	5	50
2 mL Collection Tubes	10	100
XLSM Buffer	5 mL	60 mL
SD Buffer	0.5 mL	8 mL
CPP Buffer	1.2 mL	12 mL
BBR Buffer	4 mL	40 mL
CBH Buffer	3 mL	25 mL
DW Buffer	2 mL	20 mL
EB Buffer	2 mL	30 mL
Protease Solution	150 µL	1.5 mL
2 mL bead kit 0.5 mm Glass	5	50
User Manual	✓	✓

STORAGE AND STABILITY

Protease Solution can be stored at room temperature for up to 6 months. For long-term storage, store Protease Solution at 2-8°C. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some of the buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

ILLUSTRATED PROTOCOLS



PREPARING REAGENTS

• Dilute DW Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% EtOH to be added
26-017	8 mL
26-014	60 mL

• Dilute CBH Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be added
26-017	1.2 mL
26-014	10 mL

Materials and Equipment to be Supplied by User:

• Centrifuge capable of at least 12,000 x g

• Incubator capable of 70°C

• Centrifuge Tubes with a capacity of at least 1.7 mL

• 1.5 mL centrifuge tubes for DNA storage

• Vortexer

• Ice bucket

• 100% ethanol

• 100% isopropanol

• Optional: Bead Ruptor Bead Mill Homogenizer

BEFORE STARTING:

• Prepare CBH Buffer and DW Buffer according to the "Preparing Reagents" section

• Set an incubator to 70°C

• Heat EB Buffer to 70°C

• Prepare an ice bucket

OMNI FECAL AND URINE DNA EXTRACTION INSTRUCTIONS

Fecal Extractions

1. Briefly spin the Bead Tube to remove any glass beads from the wall of the tube. Uncap the Bead Tube and save the cap for use in Step 3.
2. Add 250 µL fecal sample.
3. Add 475 µL XLSM Buffer. Seal the Bead Tube with the cap removed in Step 1.

Note: Depending on the sample type and amount, the volume of XLSM Buffer may need to be adjusted so that 300 µL can be recovered during Step 12.

Note: If only gram-negative bacteria or viral RNA & DNA is required, Steps 4-6 can be skipped.
Continue to Step 7 below.

4. Homogenize sample on a bead mill for 30-60 seconds or with continuous vortexing at maximum speed for 3-5 minutes to lyse samples.
5. Centrifuge at 1,000-2,000 x g for 15 seconds at room temperature.
6. Uncap the Bead Tube and save the cap for use in Step 8.
7. Add 72 µL SD Buffer and 20 µL Protease Solution.

Urine Extraction

1. Briefly spin the Bead Tube to remove any glass beads from the wall of the tube. Uncap the Bead Tube and save the cap for use in Step 3.
2. Add 250 µL urine sample.
3. Add 275 µL XLSM Buffer. Seal the Bead Tube with the cap removed in Step 1.

Note: Depending on the sample type and amount, the volume of XLSM Buffer may need to be adjusted so that 300 µL can be recovered during Step 12.

Note: If only gram-negative bacteria or viral RNA & DNA is required, Steps 4-6 can be skipped.
Continue to Step 7 below.

4. Homogenize sample on a bead mill for 30-60 seconds or with continuous vortexing at maximum speed for 3-5 minutes to lyse samples.
5. Centrifuge at 1,000-2,000 x g for 15 seconds at room temperature.
6. Uncap the Bead Tube and save the cap for use in Step 8.
7. Add 50 µL SD Buffer and 20 µL Protease Solution.

8. Seal the Bead Tube with the cap removed in Step 6.
9. Vortex for 60 seconds to mix thoroughly.
10. Incubate at 70°C for 15 minutes. Mix once during incubation.

Fecal Extraction

11. Centrifuge at 12,000 x g for 5 minutes.

Urine Extraction

11. Add 200 µL CPP Buffer. Let sit on ice for 5 minutes. Centrifuge at 12,000 x g for 10 minutes.

12. Transfer 300 µL cleared supernatant to a 1.5 mL centrifuge tube (not provided)

Note: Do not transfer any debris as it can reduce yield and purity.

13. Add 600 µL BBR Buffer. Vortex to mix thoroughly.
14. Let sit at room temperature for 5 minutes.
15. Insert a Micro DNA Column into a 2 mL Collection Tube.
16. Transfer 700 µL sample from Step 14 to the Micro DNA Column.
17. Centrifuge at maximum speed for 1 minute.
18. Discard the filtrate and reuse the collection tube.
19. Transfer the remaining lysate from Step 14 to the Micro DNA Column.
20. Centrifuge at maximum speed for 1 minute.
21. Discard the filtrate and reuse the collection tube.
22. Add 500 µL CBH Buffer.

Note: CBH Buffer must be diluted with 100% isopropanol before use.
See Page 4 for instructions.

23. Centrifuge at maximum speed for 30 seconds.
24. Discard the filtrate and collection tube.
25. Insert the Micro DNA Column into a new 2 mL Collection Tube.
26. Add 700 µL DW Buffer.

Note: DW Buffer must be diluted with 100% ethanol before use.
See Page 4 for instructions.

27. Centrifuge at maximum speed for 30 seconds.
28. Discard the filtrate and reuse the collection tube.
29. Repeat Steps 26-28 for a second DW Buffer wash step.
30. Centrifuge the empty Micro DNA Column at maximum speed for 2 minutes to dry the column.

Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.

31. Transfer the Micro DNA Column into a nuclease-free 1.5 mL microcentrifuge tube (not provided).
32. Add 15-100 µL EB Buffer heated to 70°C.
33. Let sit at room temperature for 2 minutes.
34. Centrifuge at maximum speed for 1 minute.
35. Repeat Steps 32-34 for a second elution step.

Note: Each 200 µL elution will typically yield of 60-70% of the DNA bound to the column. Two elutions will generally yield ~90%. However, increasing the elution volume will reduce the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µL EB Buffer which slightly reduces overall DNA yield. Volumes lower than 50 µL greatly reduce yields. In some instances, yields may be increased by incubating the column at 70°C (rather than room temperature) upon the addition of EB Buffer.

36. Store eluted DNA at -20°C

TROUBLE SHOOTING GUIDE

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at 1-800-776-4431.

Problem	Cause	Suggestion
A_{260}/A_{230} ratio is low	Salt contamination	• Repeat the DNA isolation with a new sample. • Perform a second wash with CBH Buffer.
A_{260}/A_{280} ratio is high	RNA contamination	The protocol does not remove RNA. If desired, add 5 µL RNase A (25 mg/mL) after lysate is cleared and before binding buffers are added. Let sit at room temperature for 5 minutes
Low DNA Yield or no DNA Yield	Poor homogenization of sample	Repeat the DNA isolation with a new sample, be sure to mix the sample with XLSM Buffer thoroughly. Use a Bead Ruptor possible.
	DNA washed off	Make sure CBH Buffer is mixed with isopropanol and DW Buffer is mixed with ethanol
Problems in downstream applications	BSA not added to PCR mixture	Add BSA to a final concentration of 0.1 µg/mL to the PCR mixture.
	Too much DNA inhibits PCR reactions	Dilute the DNA elute used in the downstream application if possible.
	Non-specific bands in downstream PCR	Use hot-start Taq polymerase mixture
	Inhibitory substance in the eluted DNA	Check the A_{260}/A_{230} ratio. Dilute the elute to 1:50 if necessary

NOTES

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