TISSUE DNA PURIFICATION KIT

Product Manual

26-001.....5 preps
26-007.....50 preps
26-007B.....50 preps
P/N 03-293-1 REV. D

OMNI INTERNATIONAL

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INTRODUCTION

The Omni Tissue DNA Kit is an innovative system that radically simplifies the extraction and purification of nucleic acids from a variety of sources. The key to this system is the Omni Mini DNA Column matrix that specifically, but reversibly, binds DNA or RNA allowing proteins and other contaminants to be removed. Nucleic acids are eluted with deionized water or a low salt buffer.

OVERVIEW

The Omni Tissue DNA Kit provides an easy and rapid method for the isolation of genomic DNA. Up to 30 mg of animal tissue can be readily processed. There is no need for phenol/chloroform extractions and time-consuming steps are eliminated (e.g. precipitation using isopropanol or ethanol). Purified DNA can be directly used for most applications such as PCR, Southern blotting, and restriction enzyme digestion.

Benefits of the Omni Tissue DNA Kit

- Optimized buffers that guarantee pure DNA
- No organic extractions
- Purified DNA can be directly used for most downstream applications

YIELD AND QUALITY OF DNA

Determine the absorbance of an appropriate dilution (20- to 50- fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

DNA concentration = Absorbance @ 260nm × 50 µg/mL × (Dilution Factor)

A 260/280 nm value greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) can be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations.

If necessary the DNA can be concentrated. Add sodium chloride to reach a final concentration of 0.1 M followed by 2X volumes of 100% ethanol. Mix well and incubate at -20°C for 10 minutes. Centrifuge at 10,000 x g for 15 minutes, aspirate and discard the supernatant. Add 700 µL 70% ethanol and centrifuge at 10,000 x g for 2 minutes. Aspirate and discard the supernatant, air dry the pellet for 2 minutes, and resuspend the DNA in 20 µL sterile deionized water or 10 mM Tris-HCl, pH 8.5.

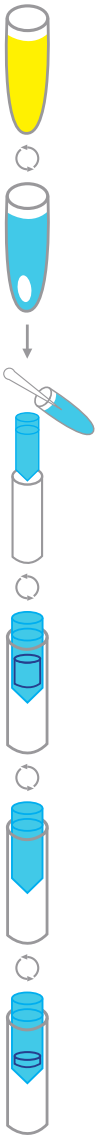
KIT CONTENTS

Product Number	26-001	26-007	26-007B
Purifications	5	50	50
Omni DNA Mini Columns	5	50	50
2 mL Collection Tubes	10	100	100
BB Buffer	5 mL	20 mL	20 mL
DLB Buffer	5 mL	20 mL	20 mL
CBH Buffer	4 mL	25 mL	25 mL
DW Buffer	1.5 mL	20 mL	20 mL
EB Buffer	2 mL	30 mL	30 mL
Protease Solution	150 µL	1.5 mL	1.5 mL
2 mL bead kit 2.8 mm ceramic	5		50
Antifoam	1 mL		1 mL
User Manual	✓	✓	✓

STORAGE AND STABILITY

Protease Solution can be stored at room temperature for 12 months. For long-term storage (>12 months), store at 2-8°C. Store all other components at room temperature (22-25°C). Check buffers for precipitates before use. Redissolve any precipitates by warming to 37˚C

ILLUSTRATED PROTOCOLS



Lyse and homogenize sample

Adjust the binding conditions

Transfer lysate into a DNA mini column to bind DNA

Wash column x 2

Dry the column

Elute DNA

BEFORE STARTING

This method is suitable for the isolation of DNA from up to 30 mg tissue. Yields vary depending on source.

Materials and equipment to be supplied by user:

- Tabletop microcentrifuge capable of 10,000 x g
- Nuclease-free 1.5 mL microcentrifuge tubes
- Shaking water baths, heat blocks, or incubators capable of 55°C and 70°C
- Bead Mill Homogenizer or Vortexer
- 100% ethanol
- Isopropanol
- Optional: RNase stock solution (100 mg/mL)
- Set water baths, heat blocks, or incubators to 55°C and 70°C.
- Prepare DW Buffer and CBH Buffer according to the directions in the "Preparing Reagents" section.
- Heat EB Buffer to 70°C.

PREPARING REAGENTS

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

Kit	100% Ethanol to be added
26-001	6 mL
26-007 and 26-007B	80 mL

- Dilute CBH Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be added
26-001	1.6 mL
26-007 and 26-007B	10 mL

- Check buffers for precipitation before use. Redissolve any precipitates by warming to 37˚C.

OMNI TISSUE DNA PURIFICATION KIT DIRECTIONS

26-007 - Tissue Digestion

1. Mince up to 30 mg tissue

Note: For samples more than 30 mg, simply scale up the volume of DLB Buffer used; for a 40-60 mg sample use 400 µL DLB Buffer.

2. Transfer to a clean 1.5 mL microcentrifuge tube (not provided)
3. Add 200 µL DLB Buffer
4. Add 25 µL Protease Solution. Vortex 10 seconds

26-007B - Tissue Homogenization

1. Mince up to 30 mg tissue and transfer to a 2 mL tube containing 2.8 mm ceramic beads.

Note: For samples more than 30 mg, simply scale up the volume of DLB Buffer used; for a 40-60 mg sample use 400 µL DLB Buffer.

2. Add 200 µL DLB Buffer and 10 uL Antifoam reagent.
3. Dissociate tissues on a bead mill. If a bead mill is not available the tissue can be processed on a vortexer at maximum speed for 10 minutes.
4. Add 25 µL Protease Solution Vortex 10 seconds.

5. Incubate at 55°C in a shaking water bath for 1 hour.

Note: Bead mill speed/power and time settings should be adjusted based on the equipment manufacturer's recommendations for the specific sample type. When using mechanical methods of homogenization, care must be taken to not over process as this could lead to DNA shearing.

Note: If a shaking water bath is not available, vortex the sample every 20-30 minutes. Lysis time depends on the amount and type of tissue used. The average time is usually less than 3 hours depending on tissue type. Lysis may need to proceed overnight or longer depending on tissue type.

Optional: Certain tissues such as liver tissue have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point if desired.

A. Add 4 µL RNase A (100 mg/mL) per 30 mg tissue (not included).

B. Let sit at room temperature for 2 minutes.

C. Proceed to Step 6 below.

6. Centrifuge at 10,000 x g for 5 minutes.

7. Transfer the supernatant to a nuclease-free 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet.

8. Add 220 µL BB Buffer. Adjust the volume of BB Buffer based on the amount of starting material. Vortex to mix thoroughly.

Example: If you used 400 µL of DLB Buffer, then add 440 µL BB Buffer and 440 µL 100% ethanol.

Note: A wispy precipitate may form upon the addition of BB Buffer. This does not interfere with DNA recovery.

9. Incubate at 70°C for 10 minutes.

10. Add 220 µL 100% ethanol. Adjust the volume of ethanol required based on the amount of starting material. Vortex to mix thoroughly.

11. Insert an Omni DNA Mini Column into a 2 mL Collection Tube.

12. Transfer the entire sample from Step 10 to the Omni DNA Mini Column including any precipitates that may have formed.

13. Centrifuge at 10,000 x g for 1 minute.

14. Discard the filtrate and reuse the collection tube.

15. Add 500 µL CBH Buffer.

Note: CBH Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

16. Centrifuge at 10,000 x g for 30 seconds.

17. Discard the filtrate and collection tube.

18. Insert the Omni DNA Mini Column into a new 2 mL Collection Tube.

19. Add 700 µL DW Buffer.

Note: DW Buffer must be diluted with ethanol before use.

t20. Centrifuge at 10,000 x g for 30 seconds.

21. Discard the filtrate and reuse the collection tube.

22. Repeat Steps 19-21 for a second DW Buffer wash step.

23. Centrifuge the empty DNA Mini Column at 10,000 x g for 2 minutes to dry the column.

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

24. Transfer the Omni DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.

25. Add 100-200 µL EB Buffer heated to 70°C.

26. Let sit at room temperature for 2 minutes.

27. Centrifuge at 10,000 x g for 1 minute.

28. Repeat Steps 25-27 for a second elution step.

Note: Each 200 µL elution will typically yield of 60-70% of the DNA bound to the column. Thus 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 at room temperature) upon the addition of EB Buffer.

29. Store eluted DNA at -20°C.

BLOOD AND BODY FLUIDS PROTOCOL

BEFORE STARTING

The procedure below has been optimized for the use with fresh or frozen blood samples of 11-250 µL in volume. Anti-coagulated blood, saliva, serum, buffy coat, or other body fluids also can be used.

Materials and equipment to be supplied by user:

- Tabletop microcentrifuge capable of 13,000 x g
- Nuclease-free 1.5 mL microcentrifuge tubes
- Shaking water baths, heat blocks, or incubators capable of 70°C
- Vortexer
- 100% ethanol
- 100% isopropanol
- **Optional:** PBS
- **Optional:** 10 mM Tris-HCl
- **Optional:** RNase A stock solution (100 mg/mL)
- Set water bath, heat block, or incubator to 70°C.
- Prepare DW Buffer and CBH Buffer according to the directions in the "Preparing Reagents" section on Pg. 4.
- Heat EB Buffer to 70°C.

OMNI BLOOD AND BODY FLUIDS KIT DIRECTIONS

26-007 - Blood and Body Fluids

1. Transfer the sample into a nuclease-free 1.5 mL microcentrifuge tube and bring the volume up to 250 µL with 10 mM Tris-HCl, PBS, or EB Buffer (provided).
2. Add 25 µL Protease Solution.
3. Add 250 µL BB Buffer. Vortex to mix thoroughly.

Note: A wispy precipitate may form upon the addition of BB Buffer. This is does not interfere with DNA recovery.

Optional: RNA will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point.

1. Add 4 µL RNase A (100 mg/mL).
2. Let sit at room temperature for 2 minutes.
3. Proceed to Step 4 below.
4. Incubate at 70°C for 10 minutes. Briefly vortex the tube once during incubation.
5. Add 250 µL 100% ethanol. Vortex to mix thoroughly.
6. Insert the Omni DNA Mini Column into a 2 mL Collection Tube.
7. Transfer the entire sample from Step 5 to the Omni DNA Mini Column including any precipitates that may have formed.
8. Centrifuge at maximum speed (≥10,000 x g) for 1 minute.
9. Discard the filtrate and reuse the collection tube.
10. Add 500 µL CBH Buffer.

Note: CBH Buffer must be diluted with 100% isopropanol before use.

11. Centrifuge at maximum speed for 30 seconds.
12. Discard the filtrate and collection tube.
13. Insert the Omni DNA Mini Column into a new 2 mL Collection Tube.
14. Add 700 µL DW Buffer. Note: DW Buffer must be diluted with 100% ethanol before use.

15. Centrifuge at maximum speed for 30 seconds.
16. Discard the filtrate and reuse the collection tube.
17. Repeat Steps 14-16 for a second DW Buffer wash step.
18. Centrifuge the empty Omni DNA Mini 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.

19. Transfer the Omni DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.
20. Add 50-200 µL EB Buffer heated to 70°C.
21. Let sit at room temperature for 2 minutes.
22. Centrifuge at maximum speed for 1 minute.
23. Repeat Steps 20-22 for a second elution step.

Note: Each 200 µL elution will typically yield of 60-70% of the DNA bound to the column. Thus 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 at room temperature) upon the addition of EB Buffer.

24. 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
Clogged Column	Incomplete lysis	Extend lysis time with DLB Buffer and Protease Solution.
	Sample size too large	If using more than 30 mg tissue, increase volumes of Protease, DLB Buffer, BB Buffer, and ethanol.
	Sample is viscious	If using more than 30 mg tissue, increase volumes of Protease, DLB Buffer, BB Buffer, and ethanol.
Low DNA yield	Incomplete homogenization	Completely homogenize sample.
	Poor elution	Repeat elution or increase elution volume. Incubation of column at 70°C for 5 minutes after addition of EB Buffer.
	Improper washing	DW Buffer must be diluted with 100% ethanol before use. CBH Buffer must be diluted with isopropanol before use.
	Overgrown Culture	Overgrown culture contains lysed cells and degraded DNA.
	Sample has low DNA content	Increase starting material and volume of all reagents (Protease, DLB Buffer, BB Buffer, ethanol) proportionally. Load aliquots of lysate through the column successively.
	Column matrix lost binding capacity during storage	Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x g for 30 seconds. Add 100 µL water to the columns and centrifuge at 10,000 x g for 30 seconds. Discard the filtrate.
Low A_{260}/A_{280} ratio	Extended centrifugation during elution	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation. It will not interfere with PCR or restriction digests.
	Poor cell lysis due to incomplete mixing with BB Buffer	Repeat the procedure, make sure to vortex the sample thoroughly with BB Buffer.

NOTES

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