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# Food DNA Isolation Kit — Product Insert

**Product # 54500**

Norgen's Food DNA Isolation Kit provides a rapid method for the isolation and purification of total DNA from a wide range of food samples originating from animals or plants. Norgen's Food DNA Isolation Kit is designed for identification of GMO-DNA or animal components in food and feed. Furthermore, the kit also provides a convenient method for the detection of food-related pathogens which may contaminate food sources. Total DNA can be purified from fresh, frozen or preserved food samples using this kit. The purified DNA is of the highest integrity, and can be used in a number of downstream applications including PCR based detection and sequencing.

**Norgen's Purification Technology**

Purification is based on spin column chromatography. The DNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process first involves grinding the food sample in a mortar with liquid nitrogen (or alternative homogenization equipment). CTAB-based Lysis Buffer L and Proteinase K are then added, followed by a short incubation at 55°C. Next, Binding Buffer I is added to the lysate followed by another short incubation on ice. The lysate is then spun in order to remove any debris. Ethanol is added to the clarified lysate, and the solution is loaded onto a spin column. Norgen's column binds nucleic acids in a manner that depends on ionic concentrations, thus only the DNA will bind to the column while most of the RNA and proteins are removed in the flowthrough. The bound DNA is then washed with the provided Buffer SK and Wash Solution A in order to remove any remaining impurities, and the purified total DNA is eluted with the Elution Buffer B. The purified DNA is of the highest integrity, and can be used in a number of downstream applications.

**Specifications**

| Kit Specifications | |
|---|---|
| Maximum Column Binding Capacity | 50 µg |
| Maximum Column Loading Volume | 650 µL |
| Maximum Amount of Starting Material:<br>Solid food material<br>Liquid sample (e.g. milk or concentrated juice) | <br>200 mg<br>1 mL to 1.5 mL |
| Time to Complete 10 Purifications | 45 minutes |

**Samples that Have Been Tested by PCR**

| | |
|---|---|
| ***Food*** | Cereal, Peach jam, Peanut butter, Chocolate, Raw and processed meat products (e.g. ham and sausage) |
| ***Pathogens Detected*** | *E. coli* O157:H7<br>*Lactobacillus* spp.<br>*Staphylococcus*<br>*Listeria monocytogenes*<br>*Salmonella enterica*<br>*Campylobacter jejuni* |

**Kit Components**

| Component | Product 54500 (50 preps) |
|---|---|
| Lysis Buffer L | 60 mL |
| Binding Buffer I | 7 mL |
| Buffer SK | 30 mL |
| Wash Solution A | 18 mL |
| Elution Buffer B | 8 mL |
| Proteinase K | 1 vial |
| Spin Columns | 50 |
| Collection Tubes | 50 |
| Elution tubes (1.7 mL) | 50 |
| Product Insert | 1 |

**Advantages**

- Fast and easy processing using a rapid spin-column format
- No hazardous chemicals required (e.g. phenol or chloroform)
- Wide compatibility with a variety of food products for GMO-DNA isolation
- Universal protocol for food related pathogen DNA isolation (Gram positive and Gram negative)

**Storage Conditions and Product Stability**

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 1 year after the date of shipment. The kit contains a ready-to-use Proteinase K, which is dissolved in a specially prepared storage buffer. The buffered Proteinase K is stable for up to 1 year after the date of shipment when stored at room temperature.

**Precautions and Disclaimers**

This kit is designed for research purposes only. This product is not intended for the diagnosis, prevention, or treatment of a disease. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

**Buffer SK** contains guanidinium salts and should be handled with care. Guanidinium salts forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

**Customer-Supplied Reagents and Equipment**

You must have the following in order to use the Food DNA Isolation Kit:

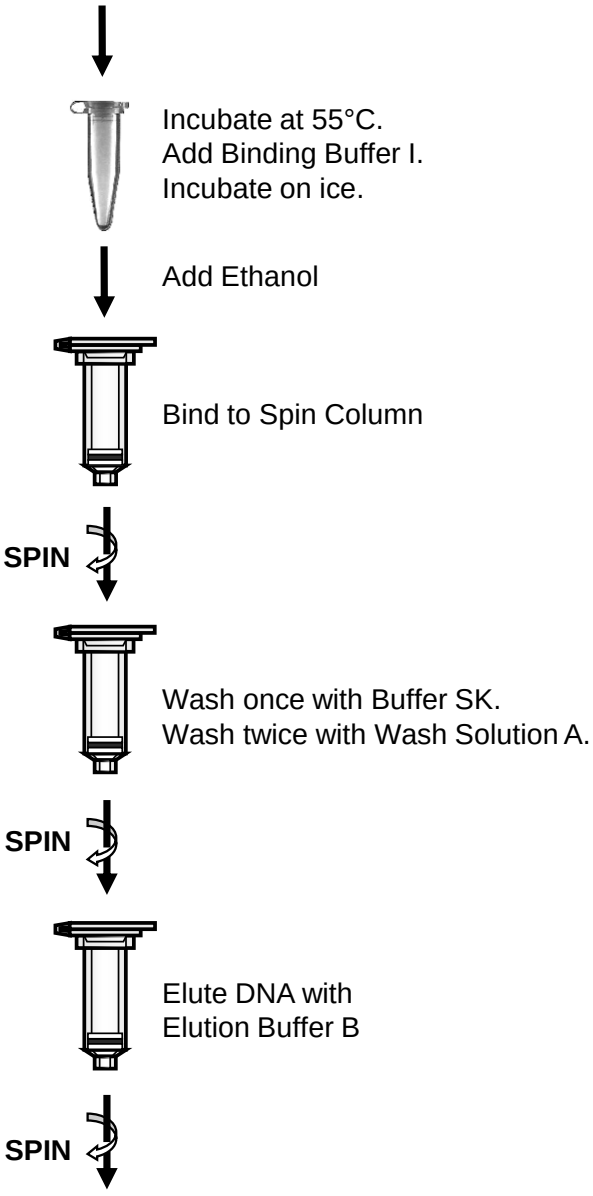
- Benchtop microcenrifuge
- 55°C Incubator
- Ice bath
- 96-100 % ethanol
- 70 % ethanol

- Liquid nitrogen or any mechanical homogenizer

## Flow Chart

Procedure for Purifying Total DNA using Norgen's Food DNA Isolation Kit

Prepare food sample by grinding with liquid nitrogen or through centrifugation**.** Add Lysis Buffer L and Proteinase K.




**Purified Total Food DNA**

# Procedures

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

***Notes Prior to Use***

- All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use. If necessary, warm to 65°C to dissolve precipitates.
- Prepare a working concentration of **Wash Solution A** by adding 42 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A.** This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Always vortex the **Proteinase K** before use.

**1. Lysate preparation**

*For food materials for GMO-DNA*

Place ≤ 200 mg of food material into a mortar that contains liquid Nitrogen and grind into a powder. Transfer the food powder to a DNase-free 1.7 mL microcentrifuge tube (not provided). Next, either perform the Optional Enzyme Incubation Step below, or proceed directly to Step 1a.

*For ketchup, sauce and other fluid samples,*

Transfer ≤ 200 mg equivalent volume to a DNase-free 1.7 mL microcentrifuge tube (not provided). Next, either perform the Optional Enzyme Incubation Step below, or proceed directly to Step 1a.

*For bacterial culture for pathogen DNA or Milk DNA*

Transfer 1 mL of milk or 1.5 mL enriched cell culture to a DNase-free 1.7 mL microcentrifuge tube (not provided) and centrifuge at 14,000 x g (~14,000 RPM) for 1 minute. Completely remove the supernatant including fat with a pipette. Next, either perform the Optional Enzyme Incubation Step below, or proceed directly to Step 1a.

**Optional enzyme incubation step:** Depending on the bacterial species this optional enzyme incubation step will increase the DNA yield. Resuspend with the suggested enzyme and incubate at 37°C for 30 minutes. **Proceed to Step 1a.**

- For Gram-negative bacteria, add 100 µL of 1 mg/mL lysozyme in TE Buffer (not provided)
- For Gram-positive bacteria, add 100 µL of 3 mg/mL lysozyme in TE Buffer (not provided)

- For *Staphylococcus* species add 5 µL of a Lysostaphin solution (2 mg/mL in water) into 100 µL of 3 mg/mL lysozyme in TE Buffer (not provided).

a. Add 750 µL (for fluid samples use 400 µL) of **Lysis Buffer L** and **20 µL of Proteinase K** (vortex before use) and vortex for 20 seconds. For the bacterial pellet, carefully resuspend with pipetting.

   **Note:** The volume of **Lysis Buffer L** can be increased depending on the sample (e.g. cereal). In that case, increase the enzyme volume proportionally.

b. Incubate at 55ºC for 30 minutes. Occasionally mix the lysate 2 or 3 times during incubation by inverting the tube.
c. Add 100 µL of **Binding Buffer I**. Mix thoroughly and incubate for 5 minutes on ice.
d. Spin for 3 minutes at **14,000 x g (~14,000 RPM)**.
e. Transfer only the clear supernatant (approximately between 650 µL and 700 µL depending on the food material) into a DNAase-free microcentrifuge tube (not provided) using a pipette. If the supernatant is less than 650 µL, adjust the volume up to 650 µL with the **Lysis Buffer L**.
f. Add an equal volume of 70% ethanol (provided by the user) to the lysate collected above (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix. **Proceed to Step 2.**

**2. Binding to Column**

a. Assemble a Spin Column with one of the provided collection tubes.
b. Apply up to 650 µL of the clarified lysate with ethanol onto the Spin Column and centrifuge for 1 minute at **10,000 × g (~10,000 RPM**). Discard the flowthrough and reassemble the spin column with the collection tube.

   **Note:** Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin at **14,000 x g (~14,000 RPM)** for an additional minute.

c. Depending on your lysate volume, repeat step **2b** if necessary.

**3. Column Wash**

a. Apply 500 µL of **Buffer SK** to the column and centrifuge for 1 minute at **10,000 × g (~10,000 RPM**).
b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Apply 500 µL of **Wash Solution A** to the column and centrifuge for 1 minute at **10,000 × g (~10,000 RPM**).
d. Discard the flowthrough and reassemble the spin column with its collection tube.
e. Repeat the step 3c and 3d.
f. Spin the column for 2 minutes at **14,000 x g (~14,000 RPM)** in order to thoroughly dry the resin. Discard the collection tube.

**4. DNA Elution**

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 100 µL of **Elution Buffer B** to the column and incubate for 1 minute at room temperature. (**NOTE:** If a higher DNA concentration is required, add 50 µL of Elution Buffer B).
c. Centrifuge for 1 minute at **10,000 x *g* (~10,000 RPM)**. If the entire volume has not been eluted, spin the column at **14,000 x g (~14,000 RPM)** for 1 additional minute.

**5. Storage of DNA**

The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.

## Troubleshooting Guide

| Problem | Possible Cause | Solution and Explanation |
|---|---|---|
| Poor DNA Recovery | Column has become clogged | Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also "Clogged Column" below. |
| | An alternative elution buffer was used | It is recommended that the Elution Buffer B supplied with this kit be used for maximum DNA recovery. |
| | 70% Ethanol was not added to the lysate | Ensure that equal amount of 70% ethanol is added to the lysate before binding to the column. |
| | Ethanol was not added to the Wash Solution A | Ensure the indicated 96 - 100% ethanol amount is added to the supplied Wash Solution A prior to use. |
| Clogged Column | Maximum amount of food material exceeds kit specifications | The optimal input is 200 mg of food material. However, for some food material 50-100 mg may be processed depending on the sample type. |
| | Centrifuge temperature too low | Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 20°C may cause precipitates to form that can cause the columns to clog. |
| DNA does not perform well in downstream applications | PCR reaction condition is needed to be optimized | Take steps to optimize the PCR conditions being used, including varying the amount of DNA template, changing the source of *Taq* polymerase, looking into the primer design and adjusting the annealing condition. |
| | Binding Buffer I was not added to the lysate | Ensure that the Binding Buffer I is added to the lysate and that it is incubated on ice for 5 minutes prior to spinning down the lysate |
| | DNA was not washed with the provided solutions | Traces of salt from the binding step may remain in the sample and salt may interfere with downstream applications. Repeat Step 3c with 500 μL of wash solution A. |
| | Ethanol carryover | Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications. |

| Related Products | Product # |
|---|---|
| Cells and Tissue DNA Isolation Kit | 53100 |
| Stool DNA Isolation Kit | 27600 |
| Bead Tubes | 26533, 26534, 26230, 26231 |
| HighRanger 1kb DNA Ladder | 11900 |
| Olive Oil DNA Isolation Kit | 61700 |
| Milk Bacterial DNA Isolation Kit | 21550 |

**Technical Support**
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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