

Food DNA Isolation Kit
Product # 54500

Product Insert

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: Solid food material Liquid sample (e.g. milk or concentrated juice)	200 mg 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	<i>E. coli</i> O157:H7 <i>Lactobacillus</i> spp. <i>Staphylococcus</i> <i>Listeria monocytogenes</i> <i>Salmonella enterica</i> <i>Campylobacter jejuni</i>

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
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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

The kit contains ready-to-use Proteinase K which is dissolved in a specially prepared storage buffer. The Proteinase K should be stored at room temperature or 4°C. All other solutions should be kept tightly sealed and 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

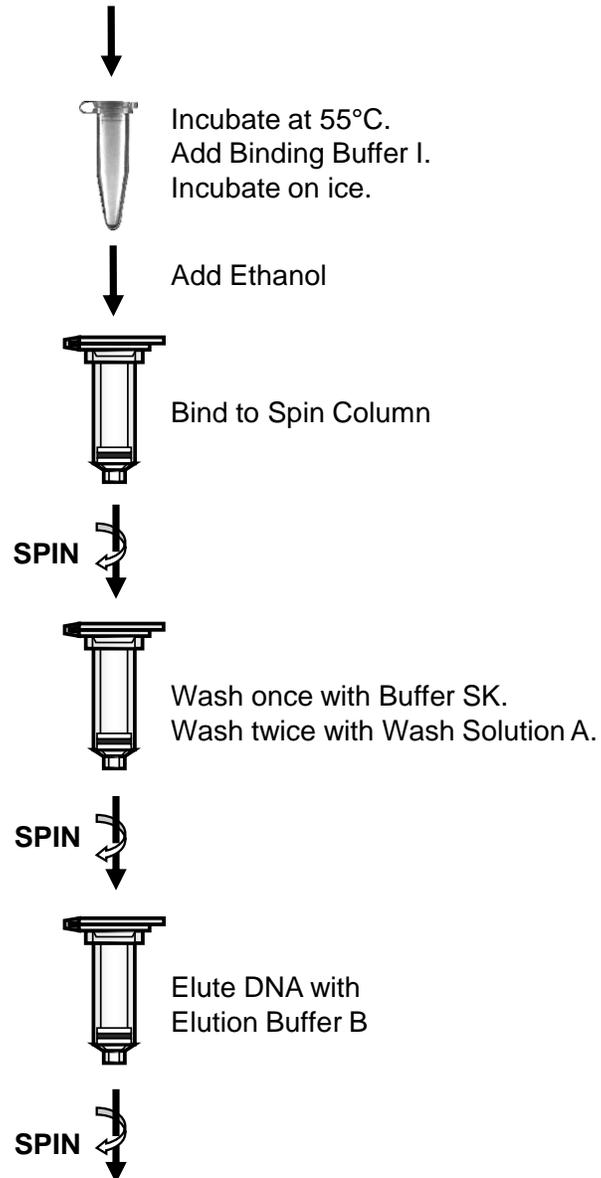
You must have the following in order to use the Food DNA Isolation Kit:

- Benchtop microcentrifuge
- 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 μ L of 1 mg/mL lysozyme in TE Buffer (not provided)
- For Gram-positive bacteria, add 100 μ L of 3 mg/mL lysozyme in TE Buffer (not provided)
- For *Staphylococcus* species add 5 μ L of a Lysostaphin solution (2 mg/mL in water) into 100 μ L 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 55°C 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 x 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 x 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 x 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 <i>Taq</i> 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 μ L 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
Milk Bacteria DNA Isolation Kit	21500, 49700
Stool DNA Isolation Kit	27600
Bead Tubes	26533, 26534, 26230, 26231
HighRanger 1kb DNA Ladder	11900

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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