Geneaid

Instruction Manual
Ver. 06.28.17
For Research Use Only

Presto™ Food DNA Extraction Kit

FGD004 (4 Preparation Sample Kit)
FGD100 (100 Preparation Kit)
FGD300 (300 Preparation Kit)

Advantages

Sample: 200 mg of raw or processed food samples
Binding Capacity: 50 µg of DNA
Format: chloroform phase separation combined with spin columns
Time: within 60 minutes
Elution Volume: 100-200 µl
Kit Storage: dry at room temperature (15-25°C)

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Geneaid Biotech Ltd.
Tel: 886 2 26960999 · Fax: 886 2 26960599 · www.geneaid.com · info@geneaid.com

Introduction

The Presto™ Food DNA Extraction Kit is designed for rapid isolation of DNA from a variety of raw and processed food samples. The unique food lysis buffer (containing nonionic detergent CTAB) when combined with Proteinase K is used to digest food tissue and proteins. PCR inhibitors such as polysaccharides and plant metabolites are removed by centrifugation. Chloroform treatment, separates residual inhibitors from the clear supernatant in the organic phase. DNA in the aqueous phase is then bound by the GD Column followed by wash and elution. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 60 minutes. The purified genomic DNA is ready for use in PCR, restriction enzyme digestion, and sequencing reactions.

Quality Control

The quality of the Presto™ Food DNA Extraction Kit is tested on a lot-to-lot basis by isolating genomic DNA from 200 mg food samples. Following the purification process, the purified DNA (A260/A280 ratio 1.8-2.0) is analyzed by electrophoresis.

Kit Components

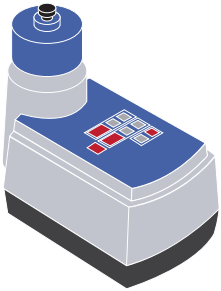
Component	FGD004	FGD100	FGD300
FD1 Buffer	10 ml	200 ml	200 ml x 3
FD2 Buffer	2 ml x 2	100 ml	100 ml x 3
Proteinase K[1] (Add ddH_2O)	1 mg (0.1 ml)	11 mg (1.1 ml)	11 mg x 3 (1.1 ml)
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer[2] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	30 ml	75 ml
GD Columns	4	100	300
2 ml Collection Tubes	4	100	300

[1]Add ddH_2O to Proteinase K (see the bottle label for volume) then vortex to ensure it is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. For extended periods, the ddH_2O and Proteinase K mixture should be stored at 4ºC. Use only fresh ddH_2O as ambient CO_2 can quickly cause acidification.

[2]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

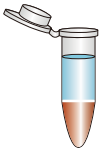
Quick Protocol Diagram



Food sample homogenization in a bead beating tube using a bead beating instrument

Add FD1 Buffer and Proteinase K then incubate at 60ºC for 30 minutes

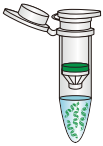
Cool the sample on ice or at 4ºC to room temperature to facilitate inhibitor precipitation



PCR inhibitor removal (Chloroform phase separation)

DNA binding to membrane while contaminants remain suspended

Wash (removal of contaminants while DNA remains bound to membrane)



Elution of pure genomic DNA which is ready for subsequent reactions

Presto™ Food DNA Extraction Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

IMPORTANT BEFORE USE!

Add ddH_2O to Proteinase K (see the bottle label for volume) then vortex to ensure it is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. For extended periods, the ddH_2O and Proteinase K mixture should be stored at 4ºC. Use only fresh ddH_2O as ambient CO_2 can quickly cause acidification.Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements

2.0 ml microcentrifuge tubes, 1.5 ml microcentrifuge tubes, standard vortex, 60ºC incubator, absolute ethanol.

Protocol Procedure

1. Sample Lysis

Homogenize **200 mg of food sample** using one of the following methods: A. Add liquid nitrogen to a mortar and grind the food samples thoroughly using a pestle. Transfer the food powder to a 2 ml centrifuge tube. B. Transfer the food samples to a 2 ml centrifuge tube containing ceramic beads or stainless steel beads then homogenize the sample with a TissueLyser, Disruptor Genie or similar. C. Transfer 200 µl of liquid samples directly to a 2 ml centrifuge tube.

Add **1 ml of FD1 Buffer and 10 µl of Proteinase K** to the microcentrifuge tube containing the homogenized food sample then vortex briefly.

NOTE: For samples which swell from liquid absorbtion (e.g., starches, cornflakes), add 2 ml of FD1 Buffer to ensure the sample is completely submerged.

Incubate the sample in a 60ºC incubator with shaking for 30 minutes. If the incubator doesn't have a shaking function, manually invert the tube every 5 minutes.

NOTE: Preheat the required Elution Buffer (200 µl per sample) to 60ºC for DNA elution.

2. PCR Inhibitor Removal

Cool the sample on ice or at 4ºC to room temperature to facilitate inhibitor precipitation. Centrifuge the tubes at 2,500 x g for 5 minutes at room temperature. Transfer **600-700 µl of clear supernatant to a clean 1.5 ml microcentrifuge tube**.

NOTE: Depending on the type of food, the supernatant may be colored. If a semi-solid layer floats on top of the supernatant, pierce it with a pipette and transfer only the clear supernatant to a clean 1.5 ml microcentrifuge tube, being careful NOT to touch any precipitate/pellet from the bottom of the tube.

Add **500 µl of Chloroform** then vortex for 15 seconds. Centrifuge at 16,000 x g for 10 minutes at room temperature. If the supernatant is not clear, centrifuge again for 5 minutes. Carefully transfer **500 µl of the upper, aqueous phase** to a new 1.5 ml microcentrifuge tube.

3. DNA Binding

Add **500 µl of FD2 Buffer** and mix IMMEDIATELY by shaking the tube vigorously for 5 seconds.

NOTE: For extracting small DNA fragments (100-200 bp) from highly processed food samples, add 1 ml of FD2 Buffer to 250 µl of the sample instead.

Place a **GD Column in a 2 ml Collection Tube**. Transfer **700 µl of sample mixture** to the GD Column then centrifuge at 16,000 x g for 1 minute at room temperature. Discard the flow-through. Place the **GD Column back in the 2 ml Collection Tube**. Transfer all of the remaining sample mixture to the GD Column then centrifuge at 16,000 x g for 1 minute at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube.

4. Wash

Add **400 µl of W1 Buffer**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer (make sure absolute ethanol was added)**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. Centrifuge at 16,000 x g for 3 minutes at room temperature to dry the column matrix.

Optional Residual Pigment Removal Step

If pigments remain on the column, perform this optional step.

Following Wash Buffer addition, add 500 µl of absolute ethanol to the GD Column. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. Centrifuge at 16,000 x g for 3 minutes at room temperature to dry the column matrix.

5. Elution

Transfer the dry **GD Column** to a new 1.5 ml microcentrifuge tube. Add **100 µl of preheated Elution Buffer**[1], TE[2] or water[3] into the CENTER of the column matrix. Let stand for at least 2 minutes to allow **Elution Buffer**, TE or water to be completely absorbed. Centrifuge at 16,000 x g for 2 minutes at room temperature to elute the purified DNA.

[1]If maximum DNA yield is required, use 200 µl of Elution Buffer (DNA concentration will be diluted). Ensure that Elution Buffer is added into the center of the GD Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GD Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the GD Column matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Troubleshooting

Low Yield

Too much starting material.

Reduce the amount of food material and/or increase the amount of FD1 Buffer.

Sample lysis or homogenization was incomplete.

Ensure that the food material is completely homogenized. Incubate the food samples and FD1 Buffer containing Proteinase K at 60ºC for 30 minutes with shaking or invert the tube every 5 minutes. Incubation time can be extended to 90 minutes if required.

Incorrect binding condition.

For extracting small DNA fragments (100-200 bp) from highly processed food samples, adding 1 ml of FD2 Buffer to 250 µl of sample lysate then mix by shaking vigorously.

Incorrect DNA elution.

Pre-heat the Elution Buffer to 60ºC prior to DNA elution. Make sure Elution Buffer is added to the center of the GD Column and is absorbed completely.

Inappropriate buffer preparation.

Add appropriate volume of absolute ethanol (see the bottle label) to the Wash Buffer prior to use.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol contamination.

Following the wash step, dry the GD Column with additional centrifugation at 16,000 x g for 5 minutes to ensure the GD Column membrane is completely dry.

PCR inhibitor contamination.

Use diluted DNA (1:10) as a template to reduce the concentration of PCR inhibitors for PCR reactions. DNA can be further purified using the GenepHlow™ PCR Cleanup Kit to eliminate PCR inhibitors.

Presto™ Food DNA Extraction Kit Functional Test Data

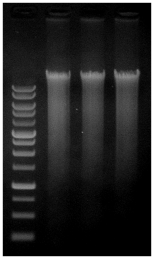


Figure 1. Total DNA was purified from 200 mg of sausage using the Presto™ Food DNA Extraction Kit. 10 µl aliquots of extracted DNA from a 100 µl eluate was analyzed on a 0.8% agarose gel. M = Geneaid™ 1 Kb DNA Ladder

Sample	ng/ul	260/280	260/230	Yield (µg)
1	95.7	1.89	2.31	9.6
2	110.4	1.90	2.30	11.0
3	108.5	1.89	2.29	10.9

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