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Introduction

E-Z 96® MagSi® Food KF DNA Kits allow rapid and reliable isolation of high-quality genomic DNA from a wide variety of Food species and tissues. Up to ninety-six 100 mg samples of wet tissue (or 30 mg dry tissue) per microplate can be processed in less than 1 hour. The system combines Omega Bio-Tek's EaZy Nucleic Acid® buffer chemistry with the convenience of MagSi® particles to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from Food tissue lysates. This kit is designed for manual or fully automated high throughput preparation of genomic, chloroplast, and mitochondrial DNA. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions, thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

Overview

If using the E-Z 96® MagSi® Food KF DNA Kit for the first time, please read this booklet to become familiar with the procedures. Dry or fresh Food tissue is disrupted and lysed in a specially formulated buffer by using lquid nitrogen or automate tissue grinder. Proteins, polysaccharides, and cellular debris are subsequently precipitated. The lysate is transferred to a 96-well microplate, binding conditions are adjusted, and genomic DNA binds to the MagSi® particles. One or two rapid wash steps remove trace contaminants such as residual polysaccharides. And finally pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the E-Z 96® MagSi® Food KF DNA Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. For long term use, store MagSi® Particles Solution at 2°C-8°C. During shipment or storage in cool ambient conditions, precipitates may form in Buffer TL. It is possible to dissolve such deposits by warming the solution at 37°C.

Kit Contents

Product	M6825-01	M6825-02	M6825-04
Purification	50 preps	200 preps	1000 preps
MagSi Particles	1.2 ml	4.5 mL	22 mL
Buffer TL	60 ml	220 mL	4 x 270 mL
Buffer SP2	20 ml	80 ml	2 x 200 ml
Lysis Buffer 3	50 ml	120 ml	2 x 250 ml
Elution Buffer	20 ml	100 mL	250 mL
RNase A	120 µL	450 µL	2.1 mL
Proteinase K	0.6 ml	2x1.2ml	12 ml
User Manual	1	1	1

NOTE: The E.Z.N.A.® MagSi Food KF DNA Isolation Kit is supplied with enough buffer for the standard protocol. However, due to increased volumes called for in some protocols, fewer preparations may be performed. Also, additional buffers can be purchased separately from Omega Bio-Tek.

Materials to be provided by user

- Centrifuge capable of at least 3,000-5,000 x g
- Rotor adapter for 96-well microplate
- Incubator equilibrated to 65°C
- Isopropanol
- 70% ethanol
- Equipment for disrupting Food tissue (MM300 Mixer Mill or Geno/Grinder 2000 and Tungsten carbide beads) or Liquid Nitrogen
- 8-or 12-channel pipette
- Reagent reservoir for pipette
- Ice Bucket
- 1.5 or 2.0 mL microcentrifuge tubes, sealed deep-well plate or capped microtube rack for sample disruption
- Kingfisher 96 or Kingfisher Flex 96 with Deep Well Magnet
- 4 Deep Well Kingfisher 96 Plates
- 1 Kingfisher 96 Plate
- 1 Tip Sleeve for Kingfisher 96 Deep Well Magnet
- Sealing Film

Kingfisher Flex 96 Food DNA Fast Protocol

This is the most robust method for isolation of total cellular (mitochondrial, chloroplast, and genomic) DNA.

Pipetting Instruction for KingFisher 96 and MagSi Food DNA protocol

Plate Type	Plate	Content	Reagent Volume
A	1	Lysate/Isopropanol/beads according to protocols below.	720 µl
A	2	70% ethanol	1000 µl
A	3	70% ethanol	1000 µl
B	4	Elution Buffer	100 µl
A	5	Tip Loading Plate	

* A= KingFisher 96 DW Plate, B=KingFisher 96 KF Plate

1. Prepare lysate by following the use instruction based on sample type.
2. Add 600 µl SPM Wash Buffer to **Plate 2.**
3. Add 600 µl SPM Wash Buffer to **Plate 3**.
4. Add 100 µl Elution Buffer to **Plate 4.**
5. Add Kingfisher 96 Tip for Deep Well Magnets Sleeve to **Plate 5.**

1. Collect 100mg ground Food Powder in a 2 ml centrifuge tube. **Add 1ml Buffer TL and 10 µL of Proteinase K.** Vortex at maxi speed for 30 seconds.

2. **Incubate the tube rack for 30-60 minutes at 65°C.** Mix sample twice during incubation by inverting tube or vortexing plate very briefly.

3. **Add 330 µL Buffer SP2 and vortex to mix**. Incubate the samples for 5 minutes on ice.

4. **Centrifuge at 3,000-12,000 x g (5,000 x g is better, if available) for 10 min.** Compact pellets will form at bottom of tubes but some particles may float. Be careful to avoid those particles during the transfer in next step.

5. Carefully transfer 400µl supernatant to a 96-well Kingfisher Deep Well Plate, making sure not to disturb the pellet or transfer any debris.

6. (Optional) Add 2µl RNase A to the sample.

7. **Add 20µl/well of MagSi® Particles Solution; follow by addition of 300µl/well of Isopropanol.**

8. Set up KingFisher Flex instrument by press "Start Key" on the Mag Bind Food DNA Protocol and load plates according to prompts from Kingfisher Unit.

9. **After the DNA isolation, seal Elution Plate which contains purified DNA with sealing Film (not provided) and store purified DNA at -20°C.**

Kingfisher Flex 96 Food DNA High Pure Protocol

This protocol is suitable for most Food samples allowing more efficient recovery of DNA. However, due to the tremendous variation in water and polysaccharide content of Foods, sample size should be limited to ≤100 mg.

Pipetting Instruction for KingFisher Flex 96 and MagSi Food DNA protocol

Plate Type	Plate	Content	Reagent Volume
A	1	Lysate /Lysis Buffer 3/Beads according to protocols below.	820 µl
A	2	70% ethanol	1000 µl
A	3	70% ethanol	1000 µl
A	4	70% ethanol	1000 µl
B	5	Elution Buffer	100 µl
A	6	Tip Loading Plate	

1. Collect 100mg ground Food Powder in a 2 ml centrifuge tube. **Add 1ml Buffer TL and 10 µL of Proteinase K.** Vortex at maxi speed for 30 seconds.

2. **Incubate the tube rack for 30-60 minutes at 65°C.** Mix sample twice during incubation by inverting tube or vortexing plate very briefly.

3. **Add 330 µL Buffer SP2 and vortex to mix.** Incubate the samples for 5 minutes on ice.

4. **Centrifuge at 3,000-12,000 x g (5,000 x g is better, if available) for 10 min.** Compact pellets will form at bottom of tubes but some particles may float. Be careful to avoid those particles during the transfer in next step.

5. **Carefully transfer 400µl supernatant to a 96-well Kingfisher Deep Well Plate,** making sure not to disturb the pellet or transfer any debris.

6. **Add 20µl/well of MagSi® Particles Solution and 400µl Lysis Buffer 3 to the sample.**

7. **Set up KingFisher Flex instrument by press "Start Key" on the Mag Bind Food DNA Protocol and load plates according to prompts from Kingfisher Unit.**

8. **After the DNA isolation, seal Elution Plate which contains purified DNA with sealing Film (not provided) and store purified DNA at -20°C.**

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl buffer, or Elution Buffer as blank. DNA concentration is calculated as:
[DNA] = ($Absorbance_{260}$) x (0.05 µg/ µL) x (Dilution factor)
The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of (A_{260}/A_{280}) of 1.7-1.9 corresponds to 85%-95% purity. Expected yields vary with both amount, and type of tissue used. 30 mg of fresh tissue will yield 10-40 µg DNA.

Troubleshooting Guide

Problem	Cause	Suggestions
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples make sure grind tissue completely.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffer TL,SP2,Lysis Buffer 3
	DNA remains bound to magnetic beads	Increase elution volume to 100 µL and incubate at 65°C for 5 min before separating eluate.
	DNA washed off.	Dilute SPM Wash Buffer by adding appropriate volume of absolute ethanol prior to use (Page 3).
Problems in downstream applications	Salt carry-over.	SPM Bufffer must be at room temperature.
	Ethanol carry-over	Dry the magnetic beads pellet completely before adding elution buffer.
DNA looks smeared on the agrose gel	DNA is disrupted by mechanic force during beads beating.	Use liquid nitrogen to disrupt sample.

Note: