

Kit Contents:

	FDK 1004 (4 preps)	FDK 1050 (50 preps)
Lysis Buffer	42 ml	250 ml × 3
Binding Buffer	5 ml	60 ml
Proteinase K (Liquid)	450 µl	1050 µl × 5
Wash Buffer (Concentrate)	1 ml *	15 ml **
Elution Buffer	1 ml	15 ml
Binding Column	4 pcs	50 pcs
Collection Tube	8 pcs	100 pcs
User Manual	1	1

Preparation of Wash Buffer by adding ethanol (96~100%) and store at RT.		
Ethanol volume for Wash Buffer	4 ml *	60 ml **

Specification:

Principle: spin column (silica membrane)
 Sample size: 200 mg or 2 g
 Operation time: <60 minutes
 Binding capacity: ≤60 µg/column
 Column applicability: centrifugation

Important Notes:

1. Homogenize the starting sample completely with a proper homogenizer and mix the sample powder evenly before the DNA extraction.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
3. Check Lysis Buffer before use. Warm Lysis Buffer at 60°C for 5 minutes if any precipitate formed.
4. Preheat shaking incubator to 60°C before the operation.
5. Add required ethanol (96~100%) to Wash Buffer before use.

Additional material provided by user:

1. Homogenizer
2. Centrifuge tube and centrifuge:
 - For 2 g sample: 50 ml centrifuge tube and centrifuge with rotor for 50 ml tube, microcentrifuge tubes (1.5 ml and 2.0 ml) and microcentrifuge.
 - For 200 mg sample: microcentrifuge tubes (1.5 ml and 2.0 ml) and microcentrifuge.
3. Ethanol (96~100%)
4. Chloroform
5. Shaking incubator (60°C)
6. Vortexer
7. Pipette and pipet tips

General Protocol: (■: for 2 g food sample; ▲: for 200 mg food sample)

Please Read Important Notes Before Starting Following Steps.

Hints: • Prepare a 60°C dry bath or water bath for step 2.

• Preheat Elution Buffer to 65°C for Elution step (Step 13).

1. ■ Transfer 2 g of powder sample to a 50 ml centrifuge tube, add 10 ml of **Lysis Buffer**, mix well by vortexing.
-**Note!** After mixing with Lysis Buffer, if the Lysis buffer is not adequate to cover the sample, add another 10 ml of Lysis Buffer and mix well by vortexing.
▲ Transfer 200 mg of powder sample to a 2 ml centrifuge tube, add 1 ml of **Lysis Buffer**, mix well by vortexing.
-**Note!** After the centrifugation (step 4), if the volume of clear supernatant is not reached to 700 µl, prepare the multiple tubes with 200 mg of sample individually to collect total 700 µl of supernatant after step 4.
2. Add ■ 100 µl ; ▲ 10 µl of **Proteinase K** and mix well by vortexing. Incubate the sample mixture at 60°C for 30 minutes and vortex the sample mixture 2~3 times during the incubation.
3. Cool down to room temperature by incubating the sample mixture on ice for 5 minutes.
4. Centrifuge at 2,500 x g for 5 minutes.
5. Transfer 700 µl of supernatant from step 4 to a 2.0 ml microcentrifuge tube.
-**Note!** In some foods, the sample mixture will form three phases after centrifugation. Transfer 700 µl totally of the middle phase to a 2.0 ml microcentrifuge tube.
6. Add 500 µl of chloroform and mix well by plus-vortexing for 15 seconds.
7. Centrifuge the sample mixture at 14,000 x g for 15 minutes.
8. Transfer 350 µl of the upper phase from step 7 to a 2.0 ml microcentrifuge tube and add 350 µl of **Binding Buffer**. mix well by plus-vortexing for 10 seconds then briefly spin the sample mixture.
9. Place a **Binding Column** to a **Collection Tube**.
10. Transfer the sample mixture from step 8 to the **Binding Column**. Centrifuge the Binding Column at 11,000 x g for 30 seconds. Discard the flow-through and place the Binding Column back to the Collection Tube
11. Add 700 µl of **Wash Buffer** (ethanol added) to Binding Column. Centrifuge at full speed (~18,000 x g) for 30 seconds. Discard the flow-through and place the Binding Column back to the Collection Tube.
-Make sure that ethanol (96~100%) has been added into Wash Buffer at the first use.
12. Centrifuge at full speed (~18,000 x g) for an additional 3 minutes to dry the Binding Column.
-Important step! This step will avoid the residual liquid to inhibit the subsequent enzymatic reactions.
13. Place the Binding Column with a 1.5 ml microcentrifuge tube. Add 100~200 µl of preheated **Elution Buffer** to the membrane center of the Binding Column. Stand the Binding Column for 1 minute at room temperature.
-Important step! For effective elution, make sure that the Elution Buffer or ddH₂O is dispensed onto the membrane center and is absorbed completely.
14. Centrifuge at full speed (~18,000 x g) for 1 minute to elute DNA.

Protocol for extraction of short DNA fragment from (■) 2 g food sample and (▲) 200 mg food sample)

Please Read Important Notes Before Starting Following Steps.

Hints: • Prepare a 60°C dry bath or water bath for step 2.

• Preheat Elution Buffer to 65°C for Elution step (Step 14).

1. ■ Transfer 2 g of powder sample to a 50 ml centrifuge tube (not provided), add 10 ml of **Lysis Buffer**, mix well by vortexing.
-**Note!** After mixing with Lysis Buffer, if the Lysis buffer is not adequate to cover the sample, add another 10 ml of Lysis Buffer and mix well by vortexing.
▲ Transfer 200 mg of powder sample to a 2 ml centrifuge tube (not provided), add 1 ml of **Lysis Buffer**, mix well by vortexing.
-**Note!** After the centrifugation (step 4), if the volume of clear supernatant is not reached to 700 µl, prepare the multiple tubes with 200 mg of sample individually to collect total 700 µl of supernatant after step 4.
2. Add ■ 100 µl ; ▲ 30 µl of **Proteinase K** and mix well by vortexing. Incubate the sample mixture at 60°C for 30 minutes and vortex the sample mixture 2~3 times during the incubation.
3. Cool down to room temperature by incubating the sample mixture on ice for 5 minutes.
4. Centrifuge at 2,500 x g for 5 minutes.
5. Transfer 700 µl of supernatant from step 4 to a 2.0 ml microcentrifuge tube.
-**Note!** In some foods, the sample mixture will form three phases after centrifugation. Transfer 700 µl totally of the middle phase to a 2.0 ml microcentrifuge tube.
6. Add 500 µl of chloroform and mix well by plus-vortexing for 15 seconds.
7. Centrifuge the sample mixture at 14,000 x g for 15 minutes.
8. Transfer 350 µl of the upper phase from step 7 to a 2.0 ml microcentrifuge tube and add 1 ml of **Binding Buffer**. mix well by plus-vortexing for 10 seconds then briefly spin the sample mixture.
9. Place a **Binding Column** to a **Collection Tube**.
10. Transfer up to 750 µl of the sample mixture from step 8 to the Binding Column. Centrifuge the Binding Column at 11,000 x g for 30 seconds. Discard the flow-through and place the Binding Column back to the Collection Tube.
11. Repeat step 10 for the rest of the sample mixture.
12. Add 700 µl of Wash Buffer (ethanol added) to Binding Column. Centrifuge at full speed (~18,000 x g) for 30 seconds. Discard the flow-through and place the Binding Column back to the Collection Tube.
-Make sure that ethanol (96~100%) has been added into Wash Buffer at the first use.
13. Centrifuge at full speed (~18,000 x g) for an additional 3 minutes to dry the Binding Column.
-Important step! This step will avoid the residual liquid to inhibit the subsequent enzymatic reactions.
14. Place the Binding Column to a 1.5 ml microcentrifuge tube. Add 100~200 µl of preheated **Elution Buffer** to the membrane center of the Binding Column. Stand the Binding Column for 1 minute at room temperature.
-Important step! For effective elution, make sure that the Elution Buffer or ddH₂O is dispensed onto the membrane center and is absorbed completely.
15. Centrifuge at full speed (~18,000 x g) for 1 minute to elute DNA.