FISHER MOLECULAR BIOLOGY

Food DNA Extraction Kit DE-090

The Food DNA Extraction kit is designed for extraction of genomic DNA from food like: meat, fish, tomato, vegetable, cereals, rice, soy , chocholate, wheat , corn , olive oil , cheese (parmiggiano, mozzarella)

Kit Contents:

	DE-090s (4 preps_sample)	**DE-090** (50 preps)
Lysis Buffer	42 ml	250 ml x 3
Binding Buffer	5 ml	60 ml
Proteinase K	1.1 mg [a] x 4	11 mg [aa] x 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

Preparation of Proteinase K solution by adding ddH2O and **Store the solution at 4 °C.**		
ddH2O volume for Proteinase K	110 µl for each tube [a]	1.1 ml for each tube [aa]

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

User Manual	1	1

Specification:

Principle: spin column (silica membrane)

Binding capacity of the column: 50 ug

Sample size : 200 mg or 2 g

DNA Yield: at least 30 ug

Operation time: < 60 min

Important Notes:

1. Homogenize the starting sample completely with a proper homogenizer and mix the sample powder before the DNA extraction.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
3. Store the Proteinase K tube at 4 °C on upon receipt of kit. And dissolve the Proteinase K with ddH2O to make a 20mg/ml stock solution and store the stock at 4 °C.
4. Check Lysis Buffer before use, Warm Lysis Buffer at 60°C for 5 minutes if any precipitate formed.
5. Preheat shaking incubator to 60 °C before the operation.
6. Preheat elution buffer to 60°C for elution step
7. Add required ethanol (96-100%) to Wash Buffer before use.
8. We suggest to use the Food Kit to use both Proteinase K and chloroform to treat the protein & lipid rich sample like cheese.The procedure is also suitable for larger sample size (up to 2 g) (mozzarella, parmiggiano, cheese /This kit is not for Milk and colostrum use Kit DE-080 Milk & Colostrum DNA Extraction Kit)

Additional material not include in the kit (provided by user):

1. Homogenizer
2. Centrifuge tube and centrifuge:
   For 2 g starting 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 starting 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. Pipets and pipet tips

For Research Use Only

General Protocol: (■: for 2 g food sample; ▲: for 200 mg food sample)
Please Read Important Notes Before Starting Following Steps.

HINT: Prepare a 60 °C dry bath or water bath for step 2.
Preheat elution buffer to 60°C for elution 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 enough to cover the sample material, add another 10 ml of Lysis Buffer and mix well by vortexing.
     ▲Transfer 200mg 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 ul, prepare the multiple tubes with 200 mg of sample individually to collect total 700 ul of supernatant after step 4.
2. Add ■ 100 ul ; ▲30 ul 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 incbuation.
3. Cool down to room temperature by incubating the sample mixture on ice for 5 minutes.
4. Centerfuge at 2,500 X g for 5 minutes.
5. Transfer 700 ul of supernatant from step 4 to a 2.0 ml micercentrifuge tube.
   - Note ! In some foods, the sample mixture will form three phases after centrifugeation. Transfer 700 ul totally of the middle phase to a 2.0 ml microcentrifuge tube.
6. Add 500 ul 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 ul of the upper phase from step 7 to a 2.0 ml microcentrifuge tube and add 350 ul 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 ul 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 when first use.
12. Centrifuge at full speed (-18,000 x g) for an additional 3 min 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** ul 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 min 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.

HINT: Prepare a 60 °C dry bath or water bath for step 2.
Preheat elution buffer to 60°C for elution 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 enough to cover the sample material, 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 ul, prepare the multiple tubes with 200 mg of sample individually to collect total 700 ul of supernatant after step 4.
2. Add ■ 100 ul ; ▲ 30 ul of Proteinase K and mix well by vortexing. Incubate the sample mixture at 60° for 30 minutes and vortex the sample mixture 2 -3 times during the incbuation.
3. Cool down to room temperature by Incubating the sample mixture on ice for 5 minutes.
4. Centerfuge at 2,500 X g for 5 minutes.
5. Transfer 700 ul of supernatant from step 4 to a 2.0 ml micercentrifuge tube.
   - Note ! In some foods, the sample mixture will form three phases after centrifugeation. Transfer 700 ul totally of the middle phase to a 2.0 ml microcentrifuge tube.
6. Add 500 ul 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 ul 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 ul 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 ul 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 when first use.
13. Centrifuge at full speed (-18,000 x g) for an additional 3 min 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 ul 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 min to elute DNA.

For Research Use Only