EasyPure® Food and Fodder Security Genomic DNA Kit

Cat. No. EE171

Storage: Carrier RNA at -20°C for one year; others at room temperature (15-25°C) for one year

Description

This kit uses modified cetyltrimethylammonium bromide (CTAB) lysis method to lysis cells. DNA is bound to high-adsorption silica-based column and eluted with elution buffer without phenol/chloroform. This kit is designed for total DNA extraction from highly processed food material due to high temperature, or/and extreme pH. It is also suitable to isolate trace amount of animal DNA from fodder. The purified DNA can be used for the detection of genetically modified organisms, animal species in food and fodder.

- Strong lysis, fast extraction
- High purity, high efficiency DNA isolation

Kit Contents

Component	EE171-01 (50 rxns)
Resuspension Buffer 13 (RB13)	180 ml
Lysis Buffer 13 (LB13)	30 ml
Precipitation Buffer 13 (PB13)	12 ml
Precipitation Buffer 14 (PB14)	18 ml
Dissolving Buffer (DB)	6 ml
Binding Buffer 13 (BB13)	10 ml
Carrier RNA (1 μg/μl)	55 μl
Clean Buffer 13 (CB13)	55 ml
Wash Buffer 13 (WB13)	12 ml
Elution Buffer (EB)	10 ml
Proteinase K (20 mg/ml)	1 ml
Genomic Spin Columns with Collection Tubes	50 each

Sample Requirement

Material	Amount
Seeds and flour	200 mg
Liquid processed food (e.g. soybean sauce, soybean milk)	20 ml
Oil (e.g. soy oil, rapeseed oil)	20 ml
Processed food (e.g. instant noodle, chips, ketchup)	200 mg
Cocoa nuts, chocolate	200 mg
Raw meat (e.g. beef, lamb, pork)	200 mg
Meat-derived processed food	200 mg
Fodder for cattle and sheep	200 mg

Reagents to be supplied by user

96-100% ethanol, isopropanol

Procedure

1) The entire procedure is carried out at room temperature.

2) Before starting, adding appropriate volume of 96-100% ethanol to BB13 and WB13.

Specification	BB13	WB13
50 rxns	15 ml	48 ml

Genomic DNA extraction from food

1. Pretreatment

1) Solid samples (e.g. instant noodle, chips, ketchup, cocoa nuts, chocolate, plant seeds, meat)

① Grind proper amount of solid food into powder (meat or plant seeds can be in liquid nitrogen). Place 200 mg of homogenized food sample into a 2 ml microcentrifuge tube.

② Add 1 ml of RB13, mix thoroughly by vortexing, incubate for 2 minutes, centrifuge at 12,000×g for 2 minutes. Discard the supernatant.

③ Repeat step ② twice.

2) Liquid samples (e.g. soybean sauce, soybean milk)

① Place 20 ml of liquid food sample into a 50 ml centrifuge tube, centrifuge at 12,000×g for 10 minutes, discard the supernatant.

② Add 1 ml of RB13 into the tube, pipetting up and down until the pellets have been completely dissolved, transfer the dissolved liquid into a 2 ml microcentrifuge tube, incubate for 2 minutes, centrifuge at 12,000×g for 2 minutes, discard the supernatant.

③ Repeat step ② twice.

3) Oil (e.g. soy oil, rapeseed oil)

- Place 20 ml of oil food sample into a 50 ml centrifuge tube, add 2 ml of RB13, mix thoroughly by vortexing, incubate for 20 minutes.
- Centrifuge at 12,000×g for 10 minutes, carefully transfer 1 ml of liqued in aqueous phase(lower phase) into a 2 ml microcentrifuge tube.

2. Add 500 µl of LB13 and 20 µl of Proteinase K (20 mg/ml) into the tube, mix thoroughly by vortexing, incubate at 55°C for no more than 1 hour. During incubation step, invert the tube at every 15 minutes.
3. Add 200 µl of PB13, mix thoroughly by vortexing, incubate on ice for 10 minutes (if RNA removal is required, add 20 µl of RNase A (10 mg/ml) before this step, mix thoroughly and incubate for 5 minutes).
4. Centrifuge at 15,000×g for 5 minutes. Carefully transfer 600 µl of supernatant into a fresh 2 ml microcentrifuge tube.
5. Add 300 µl of PB14, mix thoroughly by inverting, incubate for 5 minutes.
6. Add 1 µl of Carrier RNA and 630 µl of isopropanol, mix thoroughly by inverting (carrier RNA is not required for highly processed food).
7. Centrifuge at 15,000×g for 5 minutes, carefully discard the supernatant, save the precipitate (the precipitate may not be clearly seen for some highly processed food).
8. Add 100 µl of DB into the tube, pipetting up and down until the pellets have been completely dissolved (some pellets may be difficult to dissolve, which will not affect the following steps).
9. Add 400 µl of BB13, mix thoroughly by inverting, transfer all the liquid into a spin column. Centrifuge at 12,000×g for 30 seconds, discard the flow-through. (check to make sure that ethanol has been added into BB13 before use)
10. Add 500 µl of CB13 to the spin column, centrifuge at 12,000×g for 30 seconds, discard the flow-through.
11. Repeat step 10 once.
12. Add 500 µl of WB13 to the spin column, centrifuge at 12,000×g for 30 seconds, discard the flow-through (check to make sure that ethanol has been added into WB13 before use)
13. Repeat step 12 once.
14. Centrifuge at 12,000×g for 2 minutes, discard the flow-through.

15. Place the spin column in a sterile 1.5 ml microcentrifuge tube. Add 50 µl of EB to the column matrix. Incubate at room temperature for 2 minutes. Centrifuge at 12,000×g for 1 minute to elute the isolated genomic DNA (optional: to get more DNA by repeating this step once).

Genomic DNA extraction from fodder

1. Pretreatment

① Grind proper amount of fodder sample into powder

② Place 200 mg of homogenized fodder sample into a 2 ml microcentrifuge tube. Add 1 ml of RB13, mix thoroughly by vortexing, incubate for 2 minutes, centrifuge at 12,000×g for 2 minutes, discard the supernatant.

③ Repeat step ② twice.

2. Add 500 µl of LB13 and 20 µl of Proteinase K (20 mg/ml) into the tube, mix thoroughly by vortexing, Incubate at 55°C for 100 minutes. During incubation step, invert the tube every 20 minutes.
3. Add 200 µl of PB13, mix thoroughly by vortexing, incubate on ice for 10 minutes (if RNA removal is required, add 20 µl of RNase A (10 mg/ml) before this step, mix thoroughly and incubate for 5 minutes).
4. Centrifuge at 15,000×g for 5 minutes. Carefully transfer 600 µl of supernatant into a fresh 2 ml microcentrifuge tube.
5. Add 300 µl of PB14, mix thoroughly by inverting, incubate for 5 minutes.
6. Add 1 µl of Carrier RNA and 630 µl of isopropanol, mix thoroughly by inverting (carrier RNA is not required for highly processed food)
7. Centrifuge at 15,000×g for 5 minutes, carefully discard the supernatant, save the precipitate.
8. Add 100 µl of DB into the precipitate, pipetting up and down until precipitate completely dissolves (some precipitate may be difficult to dissolve, this would not affect subsequent experiment).
9. Add 400 µl of BB13, mix thoroughly by inverting, transfer all the liquid into a spin column. Centrifuge at 12,000×g for 30 seconds, discard the flow-through (check to make sure that ethanol has been added into BB13 before use).
10. Add 500 µl of CB13 to the spin column, centrifuge at 12,000×g for 30 seconds, discard the flow-through.
11. Repeat step 10 once.
12. Add 500 µl of WB13 to the spin column, centrifuge at 12,000×g for 30 seconds, discard the flow-through (check to make sure that ethanol has been added into WB13 before use).
13. Repeat step 12 once.
14. Centrifuge at 12,000×g for 2 minutes, discard the flow-through.
15. Place the spin column in a sterile 1.5 ml microcentrifuge tube. Add 50 µl of EB to the column matrix. Incubate at room temperature for 2 minutes. Centrifuge at 12,000×g for 1 minute to elute the isolated genomic DNA (optional: to get more DNA by repeating this step once.)

Note

If there is precipitate appearing in Lysis Buffer 13, dissolve it at 37°C water bath, mix by shaking before use.

Detection by PCR

- DNA yield from animal souce in highly processed food or fodder is low, don't use agarose gel electrophoresis or UV method to quantity the yield. We suggest to use PCR or qPCR to do it.
- Plant 18S rDNA is used as example for PCR with 2×*EasyTaq*® PCR SuperMix (+dye) (Cat. No. AS111-11) .

PCR (25 μl reaction for example)

Component	Volume	Final Concentration
Template	2 μl	as required
2×*EasyTaq*® PCR SuperMix	12.5 μl	1×
18S Forward Primer (10 μM)	0.5 μl	0.2 μM
18S Reverse Primer (10 μM)	0.5 μl	0.2 μM
ddH_2O	9.5 μl	-
Total Volume	25 μl	-

Thermal cycling conditions

94 °C	5 min	
94 °C	30 sec	35 cycles
55 °C	30 sec	
72 °C	30 sec	
72 °C	5 min	

Result Analysis

Agarose gel electrophoresis with 5-10 μl PCR products.

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