CYDN50-CHANGYU Food DNA Kit

Catalog number	Package
CYDN5001	50 preps
CYDN5002	100 preps
CYDN5003	200 preps

Storage and shipping: 15-25 °C. Stable for 12 months at room temperature. Proteinase K should be stored at 4 °C.

Description

This product uses a modified CTAB DNA extraction principle combined with a spin column to extract DNA efficiently from various foods. This manual contains one standard-fragment protocol and one small-fragment protocol; each protocol has two extraction scales (2 g for large sample amounts and 200 mg for small sample amounts). The small-fragment protocol is designed specifically for extraction of total DNA from highly processed foods. These protocols optimize the binding conditions for column purification and are recommended for strongly processed foods in which DNA has been subjected to extensive heat treatment (such as cooking or pasteurization), high pressure, irradiation, pH changes or drying, and is therefore highly fragmented (down to 100-200 bp). The small-fragment protocol provides stricter column binding conditions for small DNA fragments and has been used successfully to extract DNA from foods such as flour and corn flakes. For all other types of food and raw materials, the standard protocol should be used. The standard protocol is suitable for extraction of total DNA from complex food samples with a lower degree of processing, in which DNA is less fragmented (fragments are relatively large). DNA has been successfully extracted from foods such as tomato ketchup and chocolate. However, food samples can be very complex and the degree of processing of a particular food is not always known. It is therefore not possible to predict which protocol is best for each sample; the protocol and scale may need to be decided for each specific sample.

Kit components

Component	Storage	50 preps (CYDN5001)	100 preps (CYDN5002)	200 preps (CYDN5003)
Lysis Buffer FL	15-25 °C	250 ml × 2	250 ml × 4	250 ml × 8
Proteinase K	4 °C	1.5 ml	3 ml	6 ml
Binding Buffer PB	15-25 °C	42 ml	84 ml	168 ml
Wash Buffer WB	15-25 °C	13 ml	25 ml	50 ml
Elution Buffer EB	15-25 °C	15 ml	15 ml	20 ml
Spin Column AC	15-25 °C	50	100	200
Collection Tube	15-25 °C	50	100	200

Before first use, add absolute ethanol to Wash Buffer WB and isopropanol to Binding Buffer PB as indicated on the bottle labels and tick the boxes on the labels. The kit is stable for 12 months at room temperature.

Storage notes

Lysis Buffer FL may precipitate at low temperature. Warm in a 55 °C water bath for a few minutes to redissolve; after the buffer becomes clear, mix gently and thoroughly before use.

Avoid prolonged exposure of reagents to air, which causes evaporation, oxidation and pH changes. Keep all solutions tightly capped after use.

Notes before starting

Note: The user must supply chloroform, absolute ethanol and isopropanol.

Note: Before extraction, add absolute ethanol to the Wash Buffer WB bottle and isopropanol to the Binding Buffer PB bottle as indicated on the labels.

Note: Lysis Buffer FL may precipitate at low temperature. Warm in a 60 °C water bath for a few minutes to redissolve; after the buffer becomes clear, mix gently and thoroughly before use.

Homogenization

During the procedure, appropriate disruption of the sample material is important not only for promoting food lysis and DNA release, but also for ensuring that the starting material is homogeneous and representative of the whole food. The expected sensitivity and the sample amount must be considered. The higher the sensitivity required (for detection of trace food DNA, such as allergens or genetically modified organisms) or the more heterogeneous the food (such as chopped meat in sausage), the larger the sample amount that must be homogenized so that a representative whole sample can be transferred to the procedure. Besides sample size, the sample type also determines the homogenization procedure. These two aspects determine the most suitable homogenization device. To select the best device, determine whether the food is soft, hard or extremely hard. Several options are available for each type of food (see below).

Soft samples (for example whole fruits in fruit jams or vegetables): small amount of starting sample - small knife mill, rotor-stator homogenizer, hand blender; large amount of starting sample - large knife mill.

Solid/hard samples (for example salami or frozen foods): small amount of starting sample - small knife mill, bead mill homogenizer, mortar and pestle; large amount of starting sample - large knife mill.

Extremely solid/hard samples (for example roots or seeds): small amount of starting sample - small impact mill, bead mill homogenizer; large amount of starting sample - large impact mill.

❖ Standard-fragment protocol (2 g scale)

1. Place 2 g of homogenized food sample in a 50 ml centrifuge tube, add 10 ml Lysis Buffer FL and 25 µl proteinase K solution. Vortex briefly to ensure that the sample material is completely dispersed and wetted.

For samples that swell substantially (such as starch), double the amount of Lysis Buffer FL (to 20 ml) to ensure that enough buffer covers the sample material.

2. Incubate at 60 °C with continuous shaking for 30 minutes. To promote precipitation of inhibitors, cool the sample on ice to room temperature (15-25 °C) after incubation.

3. Centrifuge at 2,500 x g for 5 min.

The volume of supernatant depends largely on the nature of the starting material and the amount of precipitated CTAB-inhibitor complex. After centrifugation, the expected supernatant volume is 2 ml (swollen food, such as homogenized corn flakes) to 7 ml (non-swollen homogenized food, such as tomato ketchup). Do not transfer any pellet from the bottom of the tube into subsequent steps.

4. Pipette 500 µl chloroform into a 2 ml centrifuge tube. Note: chloroform is a hazardous substance; always handle it in a fume hood.

5. Carefully transfer 700 µl of the supernatant from step 3 to the tube containing chloroform. Make sure not to transfer material from the lower phase, which contains precipitated food debris.

The supernatant may be very dark in colour. Some foods may form three phases after centrifugation. If this happens, pipette through the upper phase and transfer only 700 µl of the clear middle phase. If the upper phase forms a semi-solid film (for example with chocolate), pierce the film with a pipette tip and transfer only 700 µl of the clear middle phase.

6. Vortex vigorously for 15 sec and centrifuge at 14,000 x g for 15 min.

If the supernatant is not clear, centrifuge again for 5 min.

7. Pipette 350 µl Binding Buffer PB (check that isopropanol has been added) into a new 2 ml centrifuge tube; add 350 µl of the upper aqueous phase from step 6 and vortex thoroughly.

8. Transfer the mixed liquid to Spin Column AC and centrifuge at 17,900 x g for 1 min; discard the flow-through.

9. Add 600 µl Wash Buffer WB (check that ethanol has been added), centrifuge at 17,900 x g for 30 sec and discard the flow-through.

10. Repeat the wash step once.

11. Return Spin Column AC to the empty collection tube and centrifuge at 17,900 x g for 2 min to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

12. Place Spin Column AC in a clean centrifuge tube. Apply 50-100 µl Elution Buffer EB to the centre of the membrane, leave at room temperature for 3 min and centrifuge at 17,900 x g for 1 min. Reload the eluate onto the column, leave at room temperature for 2 min and centrifuge at 17,900 x g for 1 min.

A larger elution volume gives higher elution efficiency. For a higher DNA concentration the elution volume may be reduced, but too small a volume reduces elution efficiency and DNA yield (not less than 30 µl).

For maximum yield, prewarm Elution Buffer EB in an 80-100 °C water bath before applying it to the membrane.

❖ Standard-fragment protocol (200 mg scale)

1. Place 200 mg of homogenized food sample in a 2 ml centrifuge tube (prepare at least 3-4 tubes) and add 1 ml Lysis Buffer FL and 2.5 µl proteinase K solution to each tube. Vortex briefly to ensure that the sample material is completely dispersed and wetted.

To ensure a DNA yield similar to that obtained with the standard-fragment protocol (2 g scale), prepare enough lysis tubes (at least 3-4) so that the supernatants can be combined to a volume greater than 700 µl.

For samples that swell substantially (such as starch), double the amount of Lysis Buffer FL (to 2 ml) to ensure that enough buffer covers the sample material.

2. Incubate at 60 °C with continuous shaking (1000 rpm) for 30 minutes. To promote precipitation of inhibitors, cool the sample on ice to room temperature (15-25 °C) after incubation.

3. Centrifuge at 14,000 x g for 5 min.

The volume of supernatant depends largely on the nature of the starting material and the amount of precipitated CTAB-inhibitor complex. After centrifugation, the expected supernatant volume is 200 µl (swollen food, such as homogenized corn flakes) to 700 µl (non-swollen homogenized food, such as tomato ketchup). Do not transfer any pellet from the bottom of the tube into subsequent steps.

4. Pipette 500 µl chloroform into a 2 ml centrifuge tube. Note: chloroform is a hazardous substance; always handle it in a fume hood.

5. Carefully aspirate the maximum volume of clear supernatant from each lysis tube from step 3 and transfer it to a new centrifuge tube of suitable size without disturbing the pellet. Combine the supernatants and mix by pipetting.

6. Transfer 700 µl of the combined supernatant to the tube containing chloroform.

The supernatant may be very dark in colour. Some foods may form three phases after centrifugation. If this happens, pipette through the upper phase and transfer only 700 µl of the clear middle phase. If the upper phase forms a semi-solid film (for example with chocolate), pierce the film with a pipette tip and transfer only 700 µl of the clear middle phase.

7. Vortex vigorously for 15 sec and centrifuge at 14,000 x g for 15 min.

If the supernatant is not clear, centrifuge again for 5 min.

8. Pipette 350 µl Binding Buffer PB (check that isopropanol has been added) into a new 2 ml centrifuge tube; add 350 µl of the upper aqueous phase from step 6 and vortex thoroughly.

9. Transfer the mixed liquid to Spin Column AC and centrifuge at 17,900 x g for 1 min; discard the flow-through.

10. Add 600 µl Wash Buffer WB (check that ethanol has been added), centrifuge at 17,900 x g for 30 sec and discard the flow-through.

11. Repeat the wash step once.

12. Return Spin Column AC to the empty collection tube and centrifuge at 17,900 x g for 2 min to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

13. Place Spin Column AC in a clean centrifuge tube. Apply 50-100 µl Elution Buffer EB to the centre of the membrane, leave at room temperature for 3 min and centrifuge at 17,900 x g for 1 min. Reload the eluate onto the column, leave at room temperature for 2 min and centrifuge at 17,900 x g for 1 min.

A larger elution volume gives higher elution efficiency. For a higher DNA concentration the elution volume may be reduced, but too small a volume reduces elution efficiency and DNA yield (not less than 30 µl).

For maximum yield, prewarm Elution Buffer EB in an 80-100 °C water bath before applying it to the membrane.

❖ Small-fragment protocol (2 g scale)

This protocol is designed for extraction of total DNA from large (2 g scale) fresh or processed food samples. It optimizes the spin-column binding conditions to maximize recovery of short DNA fragments. It is recommended for strongly processed foods in which DNA has been subjected to extensive heat treatment (such as cooking or pasteurization), high pressure, irradiation, pH changes or drying, and is therefore highly fragmented (down to 100-200 bp).

1. Place 2 g of homogenized food sample in a 50 ml centrifuge tube, add 10 ml Lysis Buffer FL and 25 µl proteinase K solution. Vortex briefly to ensure that the sample material is completely dispersed and wetted.

For samples that swell substantially (such as starch), double the amount of Lysis Buffer FL (to 20 ml) to ensure that enough buffer covers the sample material.

2. Incubate at 60 °C with continuous shaking for 30 minutes. To promote precipitation of inhibitors, cool the sample on ice to room temperature (15-25 °C) after incubation.

3. Centrifuge at 2,500 x g for 5 min.

The volume of supernatant depends largely on the nature of the starting material and the amount of precipitated CTAB-inhibitor complex. After centrifugation, the expected supernatant volume is 2 ml (swollen food, such as homogenized corn flakes) to 7 ml (non-swollen homogenized food, such as tomato ketchup). Do not transfer any pellet from the bottom of the tube into subsequent steps.

4. Pipette 500 µl chloroform into a 2 ml centrifuge tube. Note: chloroform is a hazardous substance; always handle it in a fume hood.

5. Carefully transfer 700 µl of the supernatant from step 3 to the tube containing chloroform. Make sure not to transfer material from the lower phase, which contains precipitated food debris.

The supernatant may be very dark in colour. Some foods may form three phases after centrifugation. If this happens, pipette through the upper phase and transfer only 700 µl of the clear middle phase. If the upper phase forms a semi-solid film (for example with chocolate), pierce the film with a pipette tip and transfer only 700 µl of the clear middle phase.

6. Vortex vigorously for 15 sec and centrifuge at 14,000 x g for 15 min.

If the supernatant is not clear, centrifuge again for 5 min.

7. Pipette 1 ml Binding Buffer PB (check that isopropanol has been added) into a new 2 ml centrifuge tube; add 250 µl of the upper aqueous phase from step 6 and vortex thoroughly.

8. Transfer 700 µl of the mixed liquid to Spin Column AC and centrifuge at 17,900 x g for 1 min; discard the flow-through. Return the column to the collection tube, transfer the remaining mixture to the column, centrifuge at 17,900 x g for 1 min and discard the flow-through.

9. Add 600 µl Wash Buffer WB (check that ethanol has been added), centrifuge at 17,900 x g for 30 sec and discard the flow-through.

10. Repeat the wash step once.

11. Return Spin Column AC to the empty collection tube and centrifuge at 17,900 x g for 2 min to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

12. Place Spin Column AC in a clean centrifuge tube. Apply 50-100 µl Elution Buffer EB to the centre of the membrane, leave at room temperature for 3 min and centrifuge at 17,900 x g for 1 min. Reload the eluate onto the column, leave at room temperature for 2 min and centrifuge at 17,900 x g for 1 min.

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For maximum yield, prewarm Elution Buffer EB in an 80-100 °C water bath before applying it to the membrane.

❖ Small-fragment protocol (200 mg scale)

This protocol is designed for extraction of total DNA from small (200 mg scale) fresh or processed food samples. It optimizes the spin-column binding conditions to maximize recovery of short DNA fragments. It is recommended for strongly processed foods in which DNA has been subjected to extensive heat treatment (such as cooking or pasteurization), high pressure, irradiation, pH changes or drying, and is therefore highly fragmented (down to 100-200 bp).

1. Place 200 mg of homogenized food sample in a 2 ml centrifuge tube (prepare at least 3-4 tubes) and add 1 ml Lysis Buffer FL and 2.5 µl proteinase K solution to each tube. Vortex briefly to ensure that the sample material is completely dispersed and wetted.

To ensure a DNA yield similar to that obtained with the standard-fragment protocol (2 g scale), prepare enough lysis tubes (at least 3-4) so that the supernatants can be combined to a volume greater than 700 µl.

For samples that swell substantially (such as starch), double the amount of Lysis Buffer FL (to 2 ml) to ensure that enough buffer covers the sample material.

2. Incubate at 60 °C with continuous shaking (1000 rpm) for 30 minutes. To promote precipitation of inhibitors, cool the sample on ice to room temperature (15-25 °C) after incubation.

3. Centrifuge at 14,000 x g for 5 min.

The volume of supernatant depends largely on the nature of the starting material and the amount of precipitated CTAB-inhibitor complex. After centrifugation, the expected supernatant volume is 200 µl (swollen food, such as homogenized corn flakes) to 700 µl (non-swollen homogenized food, such as tomato ketchup). Do not transfer any pellet from the bottom of the tube into subsequent steps.

4. Pipette 500 µl chloroform into a 2 ml centrifuge tube. Note: chloroform is a hazardous substance; always handle it in a fume hood.

5. Carefully aspirate the maximum volume of clear supernatant from each lysis tube from step 3 and transfer it to a new centrifuge tube of suitable size without disturbing the pellet. Combine the supernatants and mix by pipetting.

6. Transfer 700 µl of the combined supernatant to the tube containing chloroform.

The supernatant may be very dark in colour. Some foods may form three phases after centrifugation. If this happens, pipette through the upper phase and transfer only 700 µl of the clear middle phase. If the upper phase forms a semi-solid film (for example with chocolate), pierce the film with a pipette tip and transfer only 700 µl of the clear middle phase.

7. Vortex vigorously for 15 sec and centrifuge at 14,000 x g for 15 min.

If the supernatant is not clear, centrifuge again for 5 min.

8. Pipette 1 ml Binding Buffer PB (check that isopropanol has been added) into a new 2 ml centrifuge tube; add 250 µl of the upper aqueous phase from step 6 and vortex thoroughly.

9. Transfer the mixed liquid to Spin Column AC and centrifuge at 17,900 x g for 1 min; discard the flow-through.

10. Add 600 µl Wash Buffer WB (check that ethanol has been added), centrifuge at 17,900 x g for 30 sec and discard the flow-through.

11. Repeat the wash step once.

12. Return Spin Column AC to the empty collection tube and centrifuge at 17,900 x g for 2 min to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

13. Place Spin Column AC in a clean centrifuge tube. Apply 50-100 µl Elution Buffer EB to the centre of the membrane, leave at room temperature for 3 min and centrifuge at 17,900 x g for 1 min. Reload the eluate onto the column, leave at room temperature for 2 min and centrifuge at 17,900 x g for 1 min.

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For maximum yield, prewarm Elution Buffer EB in an 80-100 °C water bath before applying it to the membrane.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.