CYDN41-CHANGYU Fungi DNA Mini Kit

Catalog number	Package
CYDN4101	50 preps
CYDN4102	100 preps
CYDN4103	200 preps

Storage and shipping: 15-25 °C. Stable for 12 months at room temperature. RNase A should be stored at -20 °C.

Description

This kit combines a DNA spin column with a unique solution system and is suitable for rapid and simple extraction of genomic DNA from fungal tissues and cells. Purification of DNA from one or more 100 mg fresh or 20 mg dry fungal samples can be completed within 30 minutes. The procedure does not require toxic organic solvents such as phenol or chloroform and does not require time-consuming isopropanol or ethanol precipitation; polysaccharides, phenolics, enzyme inhibitors and other impurities are removed rapidly and efficiently. The purified DNA can be used directly for PCR, restriction digestion, hybridization and similar experiments.

Fresh or dry fungal tissue (cells) is ground and lysed with Lysis Buffer. Proteins, polysaccharides and cell debris are removed by precipitation. Genomic DNA then binds selectively to the silica membrane of the spin column under high-ionic-strength conditions. A series of rapid wash and centrifugation steps further removes polysaccharides, polyphenols, cell metabolites, proteins and other impurities, and purified genomic DNA is eluted from the membrane with low-salt Elution Buffer EB.

Kit components

Component	Storage	50 preps (CYDN4101)	100 preps (CYDN4102)	200 preps (CYDN4103)
RNase A (10 mg/ml)	-20 °C	250 µl	500 µl	1 ml
Buffer AP1	15-25 °C	25 ml	50 ml	100 ml
Buffer AP2	15-25 °C	10 ml	20 ml	40 ml
Buffer AP3/E	15-25 °C	15 ml	25 ml	50 ml
Wash Buffer WB	15-25 °C	15 ml	25 ml	50 ml
Elution Buffer EB	15-25 °C	15 ml	20 ml	40 ml
Spin Column AC	15-25 °C	50	100	200
Collection Tube (2 ml)	15-25 °C	50	100	200

Before first use, add the indicated volume of absolute ethanol to Buffer AP3/E and Wash Buffer WB and tick the boxes on the labels. The kit is stable for 12 months at room temperature.

Storage notes

Buffer AP1 and Buffer AP3/E may precipitate at low temperature. Warm in a 65 °C water bath for a few minutes to redissolve (AP3/E may be heated before ethanol is added but must not be heated after ethanol has been added). After the buffers become clear, cool to room temperature before use.

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

Features

The silica membrane in the spin columns is a specially manufactured adsorption membrane; adsorption varies very little from column to column and reproducibility is good.

No toxic reagents such as phenol are required, and no ethanol precipitation step is needed.

Fast and simple: a single sample is usually processed within 1 hour.

Several polysaccharide- and polyphenol-removing components and repeated column washes ensure high purity. Typical OD260/OD280 is 1.7-1.9; the DNA is suitable for PCR, Southern-blot and restriction digestion.

Notes before starting

Note: All centrifugation steps are carried out at room temperature in a conventional benchtop centrifuge reaching 13,000 rpm, such as an Eppendorf 5415C or equivalent.

Note: Preheat the required water bath to 65 °C before starting.

Note: Buffer AP3/E contains irritants. Wear latex gloves and avoid contact with skin, eyes and clothing. If skin, eyes or clothing are contaminated, rinse with plenty of water or saline.

Note: DNA yield differs between fungal materials. Typical yield from 100 mg fresh tissue is 3-25 µg.

Note: Elution Buffer EB contains no EDTA and does not interfere with downstream digestion or ligation. Water may also be used for elution if its pH is above 7.5; a low pH reduces elution efficiency, and DNA eluted with water should be stored at -20 °C. For long-term storage, DNA may be eluted with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), but EDTA may affect downstream restriction digestion, so the eluate may need to be diluted before use.

Note: Fungal species are complex and no single kit can extract DNA from every species. If a fungus is extremely rich in polysaccharides and polyphenols or has very complex secondary metabolites and this kit performs poorly, the CYDN14 CTAB Plant DNA Extraction Kit generally extracts DNA well from such samples.

❖ Procedure

Read the notes before starting. Before first use, add the indicated volume of absolute ethanol to Wash Buffer WB and Buffer AP3/E, mix thoroughly and tick the boxes on the labels.

1. Take an appropriate amount of fungal tissue and grind thoroughly to a fine powder in a mortar with liquid nitrogen.

2. Transfer the powder (100 mg fresh fungal tissue or 20 mg dry tissue) to a 1.5 ml centrifuge tube without thawing, add 400 µl Buffer AP1 and 4 µl RNase A (10 mg/ml), and vortex thoroughly to help lysis.

If the tissue is difficult to lyse, a gentle homogenization step of 10 seconds may be added. In most cases there is no need to centrifuge out incompletely lysed tissue, because a centrifugation step later removes it.

Optional: when the polysaccharide content is particularly high, add 2% PVP40 to AP1; when the polyphenol content is particularly high, add 0.2% β-mercaptoethanol to AP1. Both may be added together.

3. Incubate in a 65 °C water bath for 10 minutes, inverting the tube 2-3 times during the incubation to mix the sample.

4. Add 130 µl Buffer AP2, mix thoroughly, place on ice for 5 minutes and centrifuge at 14,000 rpm for 5-10 minutes. Carefully transfer the supernatant to a new 1.5 ml centrifuge tube without disturbing the interface material.

5. Estimate the supernatant volume and add 1.5 volumes of Buffer AP3/E (check that ethanol has been added), pipetting immediately to mix.

Note: A flocculent precipitate may appear when AP3/E is added; it does not affect DNA extraction. Add AP3/E directly to the supernatant and pipette immediately to mix.

6. Load the mixture from the previous step, including any precipitate, onto Spin Column AC placed in a collection tube. Centrifuge at 13,000 rpm for 30-60 seconds and discard the flow-through. (Load 650 µl first, centrifuge and discard the flow-through, then load the remaining solution and centrifuge again.)

7. Add 600 µl Wash Buffer WB (check that ethanol has been added), centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

8. Add 600 µl Wash Buffer WB, centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

9. Return Spin Column AC to the empty collection tube and centrifuge at 13,000 rpm for 2 minutes to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

10. Place Spin Column AC in a clean centrifuge tube. Apply 100 µl Elution Buffer EB to the centre of the membrane, leave at room temperature for 3-5 minutes and centrifuge at 12,000 rpm for 1 minute. Reload the eluate onto the column, leave at room temperature for 2 minutes and centrifuge at 12,000 rpm for 1 minute.

A larger elution volume gives higher elution efficiency; increase the elution volume if a high yield is expected and needed. For a higher DNA concentration the elution volume may be reduced, but it should not be less than 50 µl; too small a volume reduces elution efficiency and DNA yield.

Store the DNA at 2-8 °C; for long-term storage, keep at -20 °C.

Troubleshooting

Problem	Comments and suggestions
Low DNA yield	Excess starting material or incomplete lysis - use an appropriate amount of starting material and grind or homogenize thoroughly. Inappropriate binding conditions - estimate the supernatant volume accurately at step 5 and add exactly 1.5 volumes of AP3/E.
Residual RNA	Fungal RNA content in the sample is too high - increase the RNase A concentration.
No DNA obtained	Absolute ethanol was forgotten in Wash Buffer WB - add the indicated volume of absolute ethanol to WB before the first experiment.
Spin column clogged	Insufficient grinding or lysis with many clumps; the lysate is too viscous; the centrifugal force is too low - see step 2 and add a centrifugation step to remove debris; reduce the amount of starting material and do not overload; increase the centrifugal force.
Coloured eluted DNA solution or visible pigment on the membrane	Insufficient washing - after step 8, wash once more with 500 µl ethanol. Excess starting material - reduce the amount of starting material and do not overload.
Low DNA yield in the eluate	Residual ethanol in the spin column or ethanol at the bottom of the tube - make sure step 9 was performed, otherwise residual ethanol reduces elution efficiency. Water or another non-optimal liquid was used instead of Elution Buffer - read step 10 and note 5 carefully and elute only with Elution Buffer EB. The elution volume is too low - elute with 200 µl Elution Buffer.
Abnormally high A260	Silica membrane components co-eluted and interfered with absorbance - centrifuge the eluted genomic DNA again at 13,000 rpm for 1 minute and carefully use the supernatant.

DNA cannot be cut or is only partially digested by restriction enzymes	Silica membrane components co-eluted and inhibited digestion - centrifuge the eluted genomic DNA again at 13,000 rpm for 1 minute and carefully use the supernatant. Residual ethanol in the spin column or at the bottom of the tube inhibited digestion - make sure step 9 was performed, then air-dry for a few minutes to let residual ethanol evaporate.

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