CHANGYU Plant DNA Rapid Extraction Kit

Kit Contents		Cat#		
		CYDN1401	CYDN1402	CYDN1403
	Storage	50 Preps	100 Preps	200 Preps
Lysis buffer **PL**	RT	40 ml	80 ml	75 ml×2
Binding buffer **PQ**	RT	15 ml	30 ml	30 ml×2
		Add indicated ethanol before first use		
Inhibitor removal buffer **IR**	RT	25 ml	50 ml	100 ml
Washing buffer **WB**	RT	15 ml	25 ml	25 ml×2
		Add indicated ethanol before first use		
Elution buffer **EB**	RT	15 ml	15 ml	40 ml
Adsorption column **AC**	RT	50 Pieces	100 Pieces	200 Pieces
Collection tube (2ml)	RT	50 Pieces	100 Pieces	200 Pieces

Note: All reagents, when stored at the indicated temperature, are stable for 12 months

❖ Storage

1. Lysis Buffer PL or Inhibitor Removal Buffer IR may precipitate at low temperatures. If this occurs, incubate in a 55 °C water bath for several minutes until fully dissolved, and cool to room temperature before use. The concentration of guanidine hydrochloride in the binding buffer PQ is high, some precipitation may occur after adding ethanol, which does not affect the use, and the supernatant can be used directly.
2. To avoid prolonged exposure of reagents to the air to produce volatilization, oxidation, and pH value changes, each solution should be covered in time after use.

❖ Product introduction

The improved classical CTAB plant DNA isolation buffer (adding a variety of polysaccharides and polyphenol-removing components according to the characteristics of plants) rapidly lysed cells and inactivated intracellular nuclease. After chloroform extraction, the polysaccharides, polyphenols and proteins were removed by centrifugation (isopropanol was also added to the supernatant as required to centrifuge and precipitate genomic DNA to further remove other impurities). Then the genomic DNA was selectively adsorbed on the silica membrane in the spin column under the condition of chaotropic salts, and then the polysaccharides, polyphenols, cell metabolites, proteins and other impurities were further removed through a series of rapid rinsing and centrifugation. Finally, the purified genomic DNA was eluted from the silica membrane with a low-salt eluting buffer.

❖ Product Characteristics

1. All the silica membrane in the spin column are made of advanced special adsorption membranes, and the difference of adsorption capacity between the columns is very small and the reproducibility is good, ensuring highly reproducible yields and minimizing lot-to-lot variation.
2. No need of toxic phenol and without the steps of ethanol precipitation.
3. Fast, simple, and single sample operation can be generally completed in less than one hour.
4. Several kinds of polysaccharides-and-polyphenols-removing component and multiple column rinses ensure high purity. The typical ratio of OD260/OD280 is 1.7–1.9, and the length can reach 30-50 kb, which can be directly used in PCR, Southern-blot and various enzyme digestion reaction.

❖ NOTE

1. **All centrifugation steps should be performed at room temperature** using a conventional table-top centrifuge with a speed of 13,000 rpm, such as an Eppendorf 5415C or a similar centrifuge.
2. Preheat the water bath to 65 °C before the experiment.
3. Need to prepare chloroform or chloroform/isoamyl alcohol (in a volume ratio of 24:1), anhydrous ethanol, and β-mercaptoethanol.
4. Binding buffer PQ and inhibitor removal solution IR contain irritating compounds. **Wear latex gloves when handling to avoid contamination of skin, eyes and clothing. If the skin and eyes are contaminated, rinse with plenty of water or physiological saline.**
5. The amount of DNA extracted from plant tissue materials from different sources can be different, the typical output of 100mg fresh tissue is 3-25μg.
6. **The eluent EB does not contain the chelating agent EDTA,** and does not affect the downstream enzyme digestion, ligation and other reactions. **It is also possible to use water to elute, but it should be ensured that the pH is greater than 7.5** and too low pH will affect the elution efficiency. The DNA eluted with water should be stored at -20 °C. If the DNA needs to be stored for a long time, it can be eluted with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), but EDTA may affect the downstream enzyme digestion reaction and can be diluted appropriately when used.
7. **This kit is equipped with the volume of each solution according to the standard extraction process. If the content or output of DNA of the sample is low, the extraction amount needs to be expanded, and the solution needs to be purchased separately.**

❖ Standard operating procedures: (Please read the notes before the experiment)

❖ Reminders:

⇨ Before the first use, please add the specified amount of anhydrous ethanol to the washing buffer WB and the binding solution PQ, and mix well. Then please tick the box in time to mark

that ethanol has been added to avoid adding it multiple times!

⇨ Take the appropriate amount of lysate PL and preheat it at 65 °C in water bath. Add β-mercaptoethanol to a final concentration of 2% before use.

1. Take appropriate amount of plant tissue (100 mg of fresh tissue or 30 mg of dry weight tissue) and grind it into a fine powder **COMPLETELY** by adding liquid nitrogen to the mortar.

2. Transfer the fine powder to a 1.5ml centrifuge tube, do not thaw, add 600μl preheated lysate PL at 65 °C (confirm that β-mercaptoethanol has been added to 2%), mix well by vortexing gently or by pipetting up and down.

If tissue lysis is difficult, add a gentle homogenate for 10 seconds as needed to help lysis.

3. Water bath at 65 °C for 20-30 minutes, invert the tube during the water bath to mix the sample several times.

Optional steps: If the sample RNA is expected to be rich and easy to remain, 5-6μl RNase A (20mg/ml) can be added before the water bath. If the tissue is dry or the output is low, the time of water bath can be prolonged appropriately.

Note: If the residual RNA of the extracted DNA is so much that leading to smearing, distorted bands, or high background, and other abnormal electrophoresis, it is necessary to add 1% RNase A (10 mg/ml) at 37 °C or room temperature for half an hour to digest the RNA. After digestion, it can be used for PCR or enzyme digestion directly without special treatment

4. Add 700μl chloroform or chloroform/isoamyl alcohol (mixed in a volume ratio of 24:1), mix well upside down for a few minutes (or mix by vortexing), and centrifuge at 13,000 rpm for 5 minutes.

Optional step (generally not required): If the extracted plant tissue is rich in polysaccharide and polyphenols, it can be extracted with equal volume of Tris-saturated phenol (pH 8.0)/ chloroform (1:1) prior to step 4.

5. Carefully pipette the supernatant into a new 1.5 ml centrifuge tube, taking care not to pipette the boundary material.

Optional step (generally not required): If the supernatant is turbid, repeat step 4 once more until a clear supernatant is obtained.

6. Accurately estimate the amount of supernatant. Add 1.5 times the volume of the binding buffer PQ **(please check that anhydrous ethanol has been added first!)** and immediately mix well by vortexing. Precipitation may occur at this time, but it does not affect the experimental results.

7. Add the mixture obtained in the previous step (including the possible precipitation) to an adsorption column AC (the adsorption column is placed in the collection tube) and centrifuge at 13,000 rpm for 30 seconds. Discard the waste liquid in the collection tube (first add 700μl to centrifuge, discard the waste liquid, add the remaining solution, and then centrifuge again).

8. Add 500μl inhibitor removing solution IR, centrifuge at 12,000 rpm for 30 seconds, and

discard the waste liquid.

9. Add 600μl washing buffer WB **(please check that anhydrous ethanol has been added!)**, centrifuge at 12,000 rpm for 30 seconds, and discard the waste liquid.

10. Add 600μl washing buffer WB, centrifuge at 12,000 rpm for 30 seconds, and discard the waste liquid.

11. Place the adsorption column AC back into the empty collection tube and centrifuge at 13,000 rpm for 2 minutes, remove the washing buffer as completely as possible so as not to inhibit the downstream reaction with residual ethanol in the washing buffer.

12. Place the adsorption column AC in a clean centrifuge tube, add 100μl elution buffer EB to **the middle of the adsorption membrane** (preheat the elution buffer in a 65-70 °C water bath), place at room temperature for 3-5 minutes, and centrifuge at 12,000 rpm for 1 minute. Repeat once more (The resulting solution was re-added to the adsorption column and placed at room temperature for 2 minutes and centrifuged at 12,000 rpm for 1 minute).

The larger the elution volume, the higher the elution efficiency. If the expected and required output is high, the elution volume can be increased. If the required DNA concentration is high, the elution volume can be appropriately reduced, but the minimum volume should not be less than 50μl. Too small volume reduces the elution efficiency and output of DNA.

13. DNA can be stored at 2-8 °C for a short time or -20 °C for a long time.

❖ **Appendices (Operation steps for samples with low DNA content or low output):**

1. Take appropriate amount of plant tissue (400 mg fresh tissue or 200 mg dry tissue) and grind it into a fine powder completely by adding enough liquid nitrogen to the mortar.

2. Transfer the fine powder to a 15ml centrifuge tube, do not thaw, add 9ml of pre-heated lysate PL at 65 °C (confirmed that β-mercaptoethanol has been added to 2%), mix completely by vortexing and oscillating, blow with pipette tips to help lysis.

If tissue lysis is difficult, add a gentle homogenate for 10 seconds as needed to help lysis.

3. Leave at room temperature for 60 minutes, invert the centrifuge tubes from time to time to mix the sample several times.

If the tissue is dry or the output is low, it can be placed in a 65 °C water bath.

4. Add 4.5 ml chloroform/isoamyl alcohol (mixed in a volume ratio of 24:1), mix completely by vortexing, and centrifuge at 3,000g for 10 minutes.

5. Carefully pipette the supernatant into a new 15 ml centrifuge tube, taking care not to pipette the boundary material. Repeat step 4.

6. Carefully pipette the supernatant into a new 15 ml centrifuge tube, estimate the amount of supernatant, add 0.7 times the volume of isopropyl alcohol, and mix well by vortexing to precipitate the DNA.

7. Immediately precipitate the DNA by centrifugation at 3,000g for 20 minutes, discard the

supernatant, invert the open tube on the paper towel to air-dry the residual liquid, and carefully use a pipette to drain the remaining liquid around the precipitate (do not over-dry the DNA precipitate).

8. Add 300μl-400μl sterilized water preheated to 65 °C to redissolve the DNA. It may need to be incubated at 65 °C for a short time to help dissolve, during which flicking the bottom of the tube to help dissolve.

9. Add 1.5 times the volume of the binding buffer PQ (450μl-600μl, **please check first if anhydrous ethanol has been added!**), then vortex immediately and mix completely. Precipitation may occur at this time, but it does not affect the experimental results.

10. The following steps are exactly the same as the steps after the start of step 7 of the standard operation above.

This product is furnished for LABORATORY RESEARCH USE ONLY.

Not for diagnostic or therapeutic use.