DANAGENE PLANT DNA Kit

Ref. 0604.1 50 extractions
Ref. 0604.2 200 extractions

1. INTRODUCTION

1.1 Product description

This kit provides a method for efficient and rapid extraction of genomic **DNA from plant and fungal tissue and cells**.

It is known that plants contain quantities of different substances (polysaccharides, polyphenols, etc.) and that plants of the same gender or related genders may have enormous variability in their biochemical compositions, thus making it difficult to have a single method of extraction of DNA for all plants.

In order to solve this problem and to cover as many plants as possible DANAGEN-BIOTED uses a **PVP solution** which is able to bind the polysaccharides and polyphenols which are released by cell lysis and which have the ability to form complexes with nucleic acids and degrade or precipitate with them.

The procedure involves homogenizing the sample in an **Extraction Buffer** and the **PVP Solution**. Lysis is completed by incubation in a **Lysis Buffer** and **RNase** at 37°C for 30 minutes.

Cellular proteins and cell debris are removed by a **Protein precipitation buffer**, which allows the genomic DNA to be left in solution. Finally the genomic DNA is isolated by precipitation with **isopropanol**.

1.2 Kit components and storage conditions

Sufficient reagents for	50 extractions	200 extractions	Tª Stock
Extraction Buffer	25 ml	100 ml	Room temp.
PVP solution	10 ml	40 ml	4ºC
Lysis Buffer	3 ml	12 ml	Room temp.
RNasa	150 µl	600 µl	4ºC o –20ºC
Protein precipitation buffer	18 ml	72 ml	Room temp.
Hydration buffer	5 ml	20 ml	Room temp.

1.3 Equipment and reagents required and not provided

- Isopropanol.
- Ethanol 70%.
- 1.5 ml and 2.0 ml microtubes.
- 15 or 30 ml centrifuge tubes that support high speeds (for extractions greater than 100 mg of tissue).
- Microcentrifuge the clinical centrifuge.
- Vortex.
- Water bath.
- Liquid nitrogen and mortar to pulverize the tissues.
- Electric homogenizer.
- Proteinase K (20 mg/ml) for fungal extraction

1.4 Storage and stability

All components are stable for 12 months from date of purchase being stored and used as indicated.

2. PROTOCOL

2.1 PreLab Preparations

- If the **lysis solution** contains a precipitate due to the low temperatures. Incubate at 37°C and mix to dissolve the precipitate.
- Store the **RNase** at 4ºC. If the period of use of the kit is to be high, it is recommended to make aliquots and to store at -20ºC.

2.2 Preliminary considerations

- For effective DNA extraction from **plants** it is recommended to pulverize the sample in a porcelain mortar with liquid nitrogen. For soft and non-fibrous tissues, such as young leaves, flowers, etc. Can be homogenized in an electric homogenizer but always in the case of fresh samples, by dissecting the sample in small pieces, adding the **extraction buffer + PVP solution** and homogenize. For hard or fibrous tissues such as stems, seeds, etc. The use of liquid Ni is recommended.
- In the case of **fungi**, collect the mycelium from the culture by filtration, wash 3 times with sterile deionized water or PBS to remove the culture medium. Spray the tissue in a porcelain mortar with liquid Ni, store at -80°C or process the sample.
- **NOTE:** It may be necessary to vary the amount of starting material depending on the species, condition, tissue preparation or size of the genome.
- To process 100 mg samples it is necessary to use 15 or 30 ml tubes that support high centrifugation speeds.

2.3 Protocol for extracting genomic DNA from 10-20 mg of plant tissue or 10-20 mg of fungus tissue

Cellular lysis

1. Weigh the sample **10-20 mg of plant tissue or 10-20 mg of fungus tissue** and add **400 µl of Extraction Buffer + 200 µl of PVP Solution**. Homogenize with an electric homogenizer for 20 seconds.
2. Add **60 µl Lysis Solution + 3 µl RNase**. Vortex vigorously and incubate the sample at 37°C for 30 minutes. *In the case of fungi to increase the efficiency of lysis add 3 µl of **Proteinase K** (20 mg/ml) and incubate at 55°C for 1 hour or overnight. If possible, reverse or vortex periodically during incubation.*

Protein precipitation

1. Cool sample to room temperature.
2. Add **360 µl of Protein Precipitation Buffer**.
3. Vortex vigorously for 20-30 seconds.
4. Incubate **at -20°C for 10 minutes**.
5. Centrifuge **at 14,000 rpm for 5 minutes**. It will be noted that the protein precipitate forms a pellet. If particles of the pellet pass, centrifuge again in the previous section.

DNA Precipitation

1. Transfer the supernatant containing the DNA to a microtube containing **600 µl of isopropanol**. Mix by inversion several times.
2. Centrifuge **at 14,000 rpm for 3 minutes**.
3. Remove the supernatant. Add **600 µl of 70% ethanol** and invert several times to wash the pellet.
4. Centrifuge **at 14,000 rpm for 2 minutes**. Carefully remove all the **ethanol**. Watch not lose the DNA pellet. Normally this pellet has green-brown color and not the characteristic white color.
5. Invert the tube and allow drying on absorbent paper for 15 minutes.

DNA Hydration

1. Add **100 µl of the Hydration Buffer**. According to the species, the pellet size may be larger, in such cases use a greater amount of **Hydration Buffer** so that there is not an excessively viscous solution.
2. Incubate at 65°C for 1 hour with periodic stirring to aid in the dispersion of DNA.
3. Centrifuge **at 14,000 rpm for 1 minute**, as there may be particles present in the rehydrated DNA. Pass the supernatant containing the DNA to a new microtube.
4. Store at 2-8°C. For long storage, store at -20°C or -80°C.

Further removal of polysaccharides

In those species with high amounts of polysaccharides a further purification can be carried out, which usually results in a reduction in the obtained DNA of higher quality. To do this, assess whether subsequent manipulations of DNA are inhibited.

1. Adjust the hydrated DNA solution to 0.5 M **Potassium Acetate** and 30% **Ethanol**. Incubate at -20°C for 10 minutes.
2. Centrifuge at 14,000 rpm for 3 minutes.
3. Collect the supernatant with a pipette and add 1 volume of **isopropanol**.
4. Centrifuge at 14,000 rpm for 3 minutes.
5. Wash with 70% **ethanol**.
6. Centrifuge at 14,000 rpm for 2 minutes.
7. Invert the tube and allow to dry on absorbent paper for 15 minutes.
8. Resuspend the pellet in the **Rehydration Buffer**.

NOTE: To process larger samples, see the table attached with the reagents scaled according to the sample size and proceed as described protocol by applying the corresponding quantities.

Table of volumes of reagents scaled from 10 mg to 250 mg

Amount of tissue in mg	10-20	25-35	40-50	100	150	250
Tube size (ml)	1,5	2,0	15	15	15	30
Extraction buffer (ml)	0,40	0,500	0,80	1,60	2,4	4,0
PVP solution (ml)	0,20	0,250	0,40	0,80	1,20	2,0
Lysis buffer(µl)	60	75	120	240	360	600
RNasa (µl)	3	4,5	6	12	18	30
Protein precipitation buffer (ml)	0,36	0,450	0,72	1,45	2,15	3,6
Isopropanol	0,60	0,75	1,3	2,70	4,0	7,0
Hydration buffer (µl)	100	150-200	200-300	300-400	400-500	500-600

3. GUIDE TO PROBLEMS AND SOLUTIONS

Given the wide variety of samples that can be treated to extract genomic DNA with this kit, it is difficult to generalize possible problems and solutions. For this reason, we recommend that you contact the DANAGEN-BIOTED S.L technical service for any further queries regarding the working protocols or problems that may arise during the work.
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