



## DANAGENE PLANT DNA Kit

Ref. 0604.1 50 isolations

Ref. 0604.2 200 isolations

### 1. INTRODUCTION

This kit provides a method for a fast and efficient genomic **DNA extraction from cells and tissues of plants and fungi**.

It is known that plants contain different substances (polysaccharides, polyphenoles, etc.) and that plants with the same or related genus can present enormous variations in their biochemical composition. This way, it's difficult to have a single DNA extraction method for all plants.

To solve this problem and to be able to cover the biggest number of plants, this kit includes a **PVP Solution** that binds polysaccharides and polyphenoles released by the cell lyses and which have the capability of forming complexes with the nucleic acids and degrade them or to precipitate with them.

The process includes a sample homogenization in an Extraction Buffer and the PVP Solution. The lysis is completed with the incubation in a Lysis Buffer and RNase at 37°C for 30 minutes.

The cell proteins and cell debris are removed by a Protein Removal Buffer, that allows to leave the genomic DNA in solution. Finally, the genomic DNA is isolated by a precipitation with isopropanol.

### 2. KIT COMPONENTS

PRODUCT	Ref.0604.1 50 isolations	Ref. 0604.2 200 isolations	Storage Temp.
Extraction Buffer	25 ml	100 ml	Room temp.
PVP Solution	10 ml	40 ml	4°C
Lysis Solution	3 ml	12 ml	Room temp.
RNase	150 µl	600 µl	4°C / - 20°C
Protein Precipitation Buffer	18 ml	72 ml	4°C
Hydratation Buffer	5 ml	20 ml	Room temp.

#### 2.2 Equipment and additional reagents required

- ✓ Isopropanol.
- ✓ Ethanol 70%.
- ✓ 1.5 ml and 2.0 ml microtubes
- ✓ 15 or 30 ml centrifuge tubes that support high speeds (for extractions bigger than de 100 mg of tissue).
- ✓ Microcentrifuge or clinical centrifuge.
- ✓ Vortex.
- ✓ Water bath.
- ✓ Liquid nitrogen and pestle and mortar to grind tissues.
- ✓ Electronic homogenizator.
- ✓ Proteinase K (20 mg/ml) for extraction from fungi.

### 3. PROTOCOL

#### 3.1 Preliminary Preparations

- If the lysis solution contains a precipitate due to the low temperature, incubate at 37° and mix well to dissolve the precipitate.
- Store the RNase at 4°C. If you are using the kit for a long period we recommend to aliquote and store at -20°C.

#### 3.2 Preliminary Considerations

- For an effective DNA obtention from **plants** it is recommended to grind the sample a fine powder with liquid nitrogen. Soft and not fibrous tissues, like young leaves, flowers, etc. can be homogenized in an electrical homogenizer but always when you have fresh samples, dissect the sample in small pieces, add the extraction buffer together with PVP solution and homogenize. For hard or fibrous tissues, like seeds, stems, etc. It's recommended to use liquid Ni.
- In case of fungi, collect the micellium from the culture by filtration, wash 3 times with deionized sterile water or PBS to remove the culture medium. Grind the sample a fine powder with liquid nitrogen, store at -80°C or process the sample.
- **NOTE:** It can be necessary to change the starting quantity of sample depending on the specie, state, tissue preparation or genome size
- To process 100 mg of tissue it is necessary to use tubes of 15 or 30 ml for high speeds centrifugation.

#### 3.3 Protocol for the extraction of genomic DNA from 20-40 mg of plant tissue or from 10-20 mg of fungi tissue

##### • Cell Lysis

1. Weigh the sample, **20-40 mg of plant tissue or 10-20 mg of fungi 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 of Lysis Solution + 3 µl RNase**. Shake vigorously with vortex and incubate the sample at 37°C for 30 minutes. In case of fungi, to improve the lysis efficiency add *3 µl of Proteinase K (20 mg/ml) and incubate at 55°C for 1 hour or for overnight. If possible, shake with vortex or invert during the incubation.*

##### • Protein Precipitation

1. Cool the sample at room temperature.
2. Add **360 µl of Protein Precipitation Buffer**.
3. Shake vigorously with vortex for 20-30 seconds.
4. Incubate at **-20°C for 10 minutes**.
5. Centrifuge at **14.000 r.p.m. for 5 minutes**. You will be able to see that the protein precipitate forms a pellet. If particles from the pellet pass, centrifuge once again as in the previous step.

##### • DNA Precipitation

1. Pour the supernatant containing the DNA into a new microtube that contains **600 µl of isopropanol**. Mix by inverting the tube several times. Centrifuge at **14.000 r.p.m. for 3 minutes**.
2. Remove the supernatant. Add **600 µl of ethanol 70%** and invert several times to wash the pellet.
3. Centrifuge at **14.000 r.p.m. for 2 minutes**. Remove carefully all the ethanol avoiding touching the pellet.
4. Invert and drain the tube on clean absorbent paper and allow to air dry for 15 minutes.

##### • DNA Hydration

1. Add **100 µl of Hydration Buffer**. Depending on the specie, the pellet size can be bigger, in such cases use more Hydration Buffer, so you do not have an excessive viscose solution.
2. Incubate at 65°C for 1 hour, shaking periodically to help the resuspension of the DNA.
3. Centrifuge at 14.000 rpm for 1 minute as there can be particles present in the rehydrated DNA. Pour the supernatant containing the DNA into a new microtube.
4. Store at 2-8°C. For long storage periods store at -20°C o -80°C.

**NOTE:** To process bigger size samples, see chart below with the reagents scaled volumes according to the sample size and proceed as said in the protocol using the correct quantities.

**Chart of scaled reagents volume from 20mg to 500 mg**

<b>Sample quantity</b>	<b>10-20 mg</b>	<b>25-35 mg</b>	<b>40-50 mg</b>	<b>100 mg</b>	<b>15 mg</b>	<b>250 mg</b>
<b>Tube size</b>	1.5 ml	2.0 ml	15 ml	15 ml	15 ml	30 ml
<b>Extraction Buffer</b>	0.400 ml	0.500 ml	0.800 ml	1.60 ml	2.40 ml	4.0 ml
<b>PVP Solution</b>	0.20 ml	0.250ml	0.400 ml	0.800 ml	1.20 ml	2.0 ml
<b>Lysis Solution</b>	60 µl	75 µl	120 µl	240 µl	360 µl	600 µl
<b>RNase</b>	3 µl	4.5 µl	6 µl	12 µl	18 µl	30 µl
<b>Protein Precipitation Buffer</b>	0.360 ml	0.450ml	0.720 ml	1.45 ml	2.15 ml	3.6 ml
<b>Isopropanol</b>	0.60 ml	0.75 ml	1.35 ml	2.70 ml	4.0 ml	7.0 ml
<b>Hydratation Buffer</b>	100 µl	150-200 µl	200-300 µl	300-400 µl	400-500 µl	500-600 µl

#### **4. PROBLEM GUIDE AND SOLUTIONS**

Due to the great sample variety that can be treated to extract genomic DNA, it becomes difficult to generalize possible problems and answers. For this reason, we recommend to contact **DANAGEN-BIOTED** Laboratory Technical Service for any question regarding the protocols or any problem you may have during the process.