

## DANAGENE SPIN PLANT DNA KIT

Ref. 0611.1 50 isolations

Ref. 0612.2 250 isolations

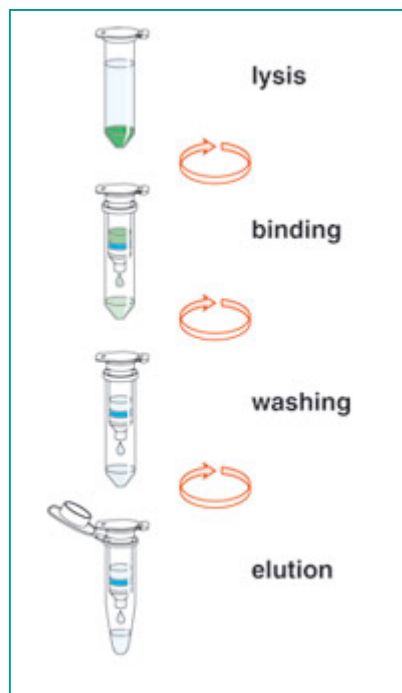
### 1.INTRODUCCION

This kit provides a method for an efficient and fast **genomic DNA extraction from tissues of plants and fungi using MiniSpin columns.**

The kit includes two optimized, alternative lysis buffers based on the established CTAB and SDS lysis methods. As plants are very heterogenous and contain a lot of different metabolites like polyphenols, polysaccharides, or acidic components, **DANAGENE SPIN PLANT DNA Kit offers two different lysis procedures for optimal processing of various samples.**

In addition we also use a **PVP solution** that can bind the polysaccharides and polyphenols.

### PROCEDURE



Plant samples are first disrupted/homogenized and then lysed in a highly optimized buffer system, containing chaotropic salt, denaturing agent and detergents. A choice of two lysis buffers based on the established CTAB or SDS method are provided.

Crude lysate are cleared by centrifugation and the cleared lysate is then mixed with the Binding Buffer and processed through a MiniSpin column containing a silica membrane to which the plant genomic DNA binds.

Contaminants and impurities such a salts, metabolites and cellular components are removal by simple washing steps with two different buffers. High-quality purified plant genomic DNA is then eluted in a low Elution Buffer. The DNA is ready-to-use for a wide variety of applications.

#### Features:

- **Silica Membrane Technology using MiniSpin columns.**
- **Choice of two optimized lysis buffers and PVP solution.**
- **High-purity DNA: typical  $A_{260}/A_{280}$  ratio 1.6 - 1.9.**
- **Plant genomic DNA isolated in 30 minutes**

#### Applications:

- **Isolation of genomic DNA from: fresh/frozen/lyophilized plant tissue and fungi.**
- **Isolated DNA is ready for downstream applications such as PCR, real-time PCR and genotyping**

## 2.COMPONENTS KIT

	<b>50 extract.</b>	<b>250 extract.</b>	<b>T<sup>a</sup> Stock</b>
<b>Lysis Buffer CTAB</b>	<b>60 ml</b>	<b>300 ml</b>	<b>Room temp.</b>
<b>Lysis/ Binding Buffer</b>	<b>35 ml</b>	<b>175 ml</b>	<b>Room temp.</b>
<b>Proteinase K <sup>(*)</sup></b>	<b>22 mg</b>	<b>105 mg</b>	<b>-20°C</b>
<b>Extraction Buffer</b>	<b>50 ml</b>	<b>250 ml</b>	<b>Room temp.</b>
<b>PVP Solution</b>	<b>10 ml</b>	<b>50 ml</b>	<b>4°C</b>
<b>Lysis Solution SDS</b>	<b>12 ml</b>	<b>60 ml</b>	<b>Room temp.</b>
<b>Precipitation Buffer</b>	<b>12 ml</b>	<b>60 ml</b>	<b>4°C</b>
<b>Desinhibition Buffer <sup>(*)</sup></b>	<b>16.5 ml</b>	<b>82. 5 ml</b>	<b>Room temp.</b>
<b>Wash Buffer <sup>(*)</sup></b>	<b>10 ml</b>	<b>50 ml</b>	<b>Room temp.</b>
<b>Elution Buffer</b>	<b>10 ml</b>	<b>50 ml</b>	<b>Room temp.</b>
<b>MicroSpin Columns</b>	<b>50 unid.</b>	<b>250 unid.</b>	<b>Room temp.</b>
<b>Collection tubes</b>	<b>100 unid.</b>	<b>500 unid.</b>	<b>Room temp.</b>

### 2.2 Equipment and additional reagents required

- ✓ Isopropanol.
- ✓ Ethanol 70%.
- ✓ 1.5 ml and 2.0 ml microtubes
- ✓ Microcentrifuge .
- ✓ Vortex.
- ✓ Water bath.
- ✓ Liquid nitrogen and pestle and mortar to grind tissues.
- ✓ Electronic homogenizator.

### 3. PROTOCOL

#### 3.1 Preliminary Preparations

- Both the Lysis/ Binding Buffer and the Desinhibition Buffer contain Guanidine hydrochloride which is an irritant agent, for this reason we recommend to use gloves and glasses for its manipulation.
- Dissolve the proteinase K in **1.1 ml** (50 extractions) or in **5.2 ml** (250 extractions) of nuclease-free water and store at  $-20^{\circ}\text{C}$ . It is recommended to do several aliquots to avoid many thaw/freeze cycles. At this temperature it is stable for 1 year.
- Verify that the Lysis Buffer SDS or the Lysis/ Binding Buffer do not have precipitates due to the low temperatures. If necessary, dissolve heating at  $37^{\circ}\text{C}$ .
- Add **10 ml** (50 extraction) or **50 ml** (250 extractions) of Ethanol 100 % to the Desinhibition Buffer. Keep the container closed to avoid the ethanol evaporation.
- Add **40 ml** (50 extractions) or **200 ml** (250 extractions) of Ethanol 100 % to the Wash Buffer. Keep the container closed to avoid the ethanol evaporation.
- Pre-heat the Elution Buffer at  $70^{\circ}\text{C}$ .

#### 3.2 Preliminary Considerations

- **It can be necessary to reduce the starting quantity of sample depending on the specie, state, tissue preparation or genome size.**
- **The first time that one works with a sample of plant tissue, we recommend to use the 2 isoaltion methods (A and B) for establishing which is the method that works better with this certain sample.**

#### METHOD A. Based on Lysis Solution SDS

1. Weigh **180-200 mg of plant tissue**. Add **1000  $\mu\text{l}$  of Extraction Buffer + 200  $\mu\text{l}$  PVP solution** and place them in 2.0 ml microtube. Vortex vigorously. Homogenize with an electric homogenizator for 20 seconds.

For an effective DNA isolation from **plant tissues**, 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 homogenizator 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  $-80^{\circ}\text{C}$  or process the sample.

2. Add **200  $\mu\text{l}$  Lysis Solution SDS. Incubate at  $80^{\circ}\text{C}$  for 30-45 minutes.** If possible, shake with vortex or invert during the incubation. Verify that the Lysis Buffer SDS do not have precipitates due to the low temperatures. If necessary, dissolve heating at  $37^{\circ}\text{C}$ .

3. **Centrifuge at 14.000 rpm for 5 minutes.**

4. Pour the supernatant to a new 2.0 ml microtube with **200 µl Precipitation Buffer**. Incubate at  $-20^{\circ}\text{C}$  for 3 minutes.

**5. Centrifuge at 14.000 rpm for 5 minutes.** A pellet will appear and in the surface a layer, to introduce the pipette tip crossing this superficial layer, only trying to pick up **600-700 µl of supernatant** that it is the transparent liquid with color (to avoid to catch pellet and superficial layer) and to place in a 2.0 ml microtube.

6. Add **600 µl-700 Lysis/ Binding Buffer + 20 µl Proteinase K to 600-700 µl of supernatant**. Mix well. **Incubate at  $70^{\circ}\text{C}$  for 10 minutes.**

7. Add **300 µl of Isopropanol**. Mix well.

8. Pipette **750 µl** the lysate into reservoir of a combined MicroSpin Column – collection tube assembly. **Centrifuge at 12.000 rpm for 60 seconds.** Remove the collection tube.

9. To repeat the point N.8 with the **750 µl** remaining.

10. Place the MicroSpin column in a new collection tube and add **500 µl of Desinhibition Buffer** to the reservoir. **Centrifuge at 10.000-12.000 rpm for 60 seconds.** Remove the liquid.

**11. Add 500 µl of Wash Buffer** into reservoir of MicroSpin column. **Centrifuge at 14.000 rpm for 60 seconds.** Remove the liquid.

**12. 2° Wash. Add 500 µl of Wash Buffer** into reservoir of MicroSpin column. **Centrifuge at 14.000 rpm for 60 seconds.** Remove the liquid.

**13. Centrifuge at maximum speed for 2 minutes to remove the residual ethanol.**

14. Remove the collection tube and insert the MicroSpin column in a 1.5 ml microtube. **Add 50-100 µl of Elution Buffer** (preheated at  $70^{\circ}\text{C}$ ) into reservoir of MicroSpin column. Incubate 1 minute.

**15. Centrifuge at maximum speed for 60 seconds.**

**16. Add another 50-100 µl of Elution Buffer** (preheated at  $70^{\circ}\text{C}$ ) into reservoir of MicroSpin column. Incubate 1 minute.

**17. Centrifuge at maximum speed for 60 seconds.** The microtube contains now genomic DNA.

## **METHOD B. Based on Lysis Buffer CTAB**

1. Weigh **180-200 mg of plant tissue**. Add **1200 µl Lysis Buffer CTAB** and place them in 2.0 ml microtube . Vortex vigorously . Homogenize with an electric homogenizator for 20 seconds.

For an effective DNA isolation from **plant tissues**, 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 homogenizator but always when you have fresh samples, dissect the sample in small pieces , add the Lysis Buffer CTAB 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  $-80^{\circ}\text{C}$  or process the sample.

**2. Incubate at 80°C for 30-45 minutes.** If possible, shake with vortex or invert during the incubation.

**3. Centrifuge at 14.000 rpm for 10 minutes.** A pellet will appear and in the surface a layer of fat, to introduce the pipette tip crossing this superficial layer of fat, only trying to pick up **700 µl of supernatant** that it is the transparent liquid with color (to avoid to catch pellet and superficial layer) and to place in a 2.0 ml microtube.

4. Add **700 Lysis/ Binding Buffer + 20 µl Proteinase K to 700 µl of supernatant**. Mix well.. **Incubate at 70°C for 10 minutes.**

5. Add **300 µl of Isopropanol**. Mix well.

6. Pipette **750 µl** the lysate into reservoir of a combined MicroSpin Column – collection tube assembly. **Centrifuge at 12.000 rpm for 60 seconds.** Remove the collection tube.

7. To repeat the point N.6 with the **750 µl** remaining .

8. Place the MicroSpin column in a new collection tube and add **500 µl of Desinhibition Buffer** to the reservoir. **Centrifuge at 10.000-12.000 rpm for 60 seconds.** Remove the liquid.

**9. Add 500 µl of Wash Buffer** into reservoir of MicroSpin column. **Centrifuge at 14.000 rpm for 60 seconds.** Remove the liquid.

**10. 2° Wash.** Add **500 µl of Wash Buffer** into reservoir of MicroSpin column. **Centrifuge at 14.000 rpm for 60 seconds.** Remove the liquid.

**11. Centrifuge at maximum speed for 2 minutes to remove the residual ethanol.**

12. Remove the collection tube and insert the MicroSpin column in a 1.5 ml microtube. **Add 50-100 µl of Elution Buffer** (preheated at 70°C) into reservoir of MicroSpin column. Incubate 1 minute.

**13. Centrifuge at maximum speed for 60 seconds.**

**14. Add another 50-100 µl of Elution Buffer** (preheated at 70°C) into reservoir of MicroSpin column. Incubate 1 minute.

**15. Centrifuge at maximum speed for 60 seconds.** The microtube contains now genomic DNA.

#### **4. PROBLEM GUIDE AND POSSIBLE ANSWER**

For any doubt or additional consultation on the protocol, do not hesitate to contact with the technical service of DANAGEN-BIOTED S.L [info@danagen.es](mailto:info@danagen.es)