



chemagic DNA Plant Kit

for 100 preparations

for research only

Kit Components

Magnetic Beads	3 ml
Lysis Buffer 1	40 ml
Binding Buffer 2	32 ml
Wash Buffer 3	90 ml
Wash Buffer 4	90 ml
Wash Buffer 5	100 ml
Elution Buffer 6	20 ml

The **Elution Buffer** is 10 mM Tris-HCl pH 8.0.

This kit contains enough materials for 100 isolations from 20 - 50 mg plant material and is optimized for use with **chemagic** Magnetic Separators.

Required Materials

- Magnetic Separator
- 70 % Ethanol
- RNase A (100 mg/ml)

Storage Conditions and Safety Information

This kit may be stored at room temperature (15 – 25 °C) and is stable for at least 1 year following delivery. The kit buffers contain irritant substances. Take appropriate laboratory safety measures and wear gloves when handling. Lysis buffer contains CTAB which is dangerous to the aquatic or nonaquatic environment and should be disposed in accordance with state, federal and local regulations and requirements.

UV Measurements

In some cases there may be traces of the magnetic beads left in the eluate after removal from the tube. Such particles will not interfere with PCR and most downstream applications but may increase the background in UV measurements. In such a case, prior to UV analysis, we recommend an additional application of the magnet to the eluate for 3 minutes in order to separate any traces of particles. For pure DNA the expected A_{260}/A_{280} ratio is between 1.8 - 1.9. The A_{260} value should fall between 0.1 and 1.0 for accurate readings.



Purification Protocol for DNA from 20 - 50 mg Plant Material

Disrupt and homogenize 20 – 50 mg plant material using one of the following methods

1a. **Bead-Mill**

(eg Retsch, Geno/Grinder 2000, FastPrep) or rotor-stator (eg Ultraturrax)

Add 400 µl **Lysis Buffer 1** and 2 µl **RNase A** (100mg/ml) to the sample in either a 96 deep well plate or a 2 ml screw cap tube and homogenize according to the instrument supplier's instructions. Times and speed have to be determined for each sample type.

1b. **Mortar and Pestle**

Thoroughly grind the sample in liquid nitrogen to obtain a fine powder.

Add 400 µl **Lysis Buffer 1** and 2 µl **RNase A** (100mg/ml) to the ground powder in the tube and mix thoroughly.

2. Add 30 µl resuspended **Magnetic Beads** and 320 µl **Binding Buffer 2**. Mix with 6 pipetting strokes and incubate 5 minutes at room temperature.
3. Following incubation, place the tube in a Magnetic Separator to draw the beads to the side of the tube for 2 minutes. Pipette off the supernatant and then remove the tube from the magnet.
4. Add 900 µl **Wash Buffer 3** to the tube. Resuspend the beads in the wash buffer by pipetting the bead pellet up and down 15 times and incubate for one minute. Separate the beads using the Magnetic Separator and discard supernatant.
5. Repeat the washing procedure using 900 µl **Wash Buffer 4**.
6. Repeat the washing procedure using 900 µl **70% ethanol**.
7. Separate the beads magnetically and remove the supernatant. Then, while leaving the tube in the Magnetic Separator, and the beads attracted to the side of the tube, gently add 1 ml **Wash Buffer 5**, being careful not to disrupt the pellet. Pipette off all **Wash Buffer 5** one minute after addition.
8. Add 200 µl (or another suitable volume) of **Elution Buffer 6** to the tube and resuspend the beads by pipetting.
9. Incubate the suspension for 10 minutes at 55 °C, with gentle agitation to facilitate complete DNA elution.
10. Following DNA elution place the tube in the Magnetic Separator for one minute to separate all the beads from solution. Transfer the eluate containing the purified Nucleic Acids to a clean tube.