

BILATEST Plant Kit



100 Extractions

Product description

This kit is designed for the fast and easy DNA purification from plants. It contains enough materials for 100 isolations from 20-50 mg plant material.

The complete protocol takes approximately 30 minutes (without lysis), the expected yield from 20 mg plant material is 5-30 µg total DNA (depending on plant species).

Included reagents

Reagent	Volume
(1) Magnetic Beads	3 ml
(2) Lysis Buffer	40 ml
(3) Binding Buffer	32 ml
(4) Washing Buffer A	90 ml
(5) Washing Buffer B	90 ml
(6) Washing Buffer C	100 ml
(7) Elution Buffer	20 ml

The **Elution Buffer (7)** is 10 mM Tris-HCl, pH 8.0.

Required Materials

- ? Magnetic Particle Separator
- ? 70% EtOH
- ? RNase A (100 mg/ml)

This kit is optimized for use with BILATEC Magnetic Separators (e.g. BILATEST magnetic separator M 12+12 for 1.5ml tubes, Order-No. 210141).

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 non-aquatic 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 be 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 (2)** and **2 µl RNase A** (100 mg/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 (2)** and 2 µl **RNase A** (100 mg/ml) to the ground powder in the tube and mix thoroughly.

2. Add **30 µl** resuspended **Magnetic Beads (1)** and **320 µl Binding Buffer (3)**. 6 pipetting strokes and incubate 5 minutes at room temperature.
3. Following incubation, place the tube in a Magnetic Separator to draw the **Magnetic Bead/DNA Complex** to the side of the tube. Leave 2 minutes, then discard the supernatant and remove the tube from the magnet position.
4. Add **900 µl Washing Buffer A (4)** to the tube and thoroughly resuspend the beads in the washing buffer by pipetting the bead pellet up and down 15 times. Incubate for one minute and separate the beads using the Magnetic Separator and discard supernatant.
5. Repeat the washing procedure using **900 µl Wash Buffer B (5)**.
6. Repeat the washing procedure using **900 µl 70% EtOH**.
7. With the tube in the Magnetic Separator and the beads attracted to the side of the tube gently add **1 ml** (or as large a volume as possible) **Wash Buffer C (6)**, **being careful not to disrupt the pellet**. Leave 60 seconds without resuspending the bead pellet and then carefully remove and discard the supernatant.
(**Note:** resuspension of the bead pellet in **Wash Buffer C (6)** may reduce the final DNA yield.)
8. Add **200 µl** (or another suitable volume) of **Elution Buffer (7)** to the tube and thoroughly resuspend the **Magnetic Bead/DNA Complex** by pipetting the pellet up and down 10 to 15 times.
9. Incubate the suspension for **10 minutes at 55°C** with agitation (1000 rpm) to facilitate complete DNA elution.
10. Following DNA elution place the tube in the Magnetic Separator for 2 minutes or until all the **Magnetic Beads (1)** have separated from the eluate. Transfer the eluate containing the purified DNA to a clean tube.