# Plant DNA extraction Kit

# (100 rxns)

Zellbio GmbH (Germany)

CAT No. ZX-22103-100

www.zellx.de

**For use with PCR, real-time PCR, Southern Blotting and RFLP**

**!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!**

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Please read this insert completely prior to using the product.


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## Background

ZellX® Plant DNA extraction Kit has been designed for the quick purification of total DNA from a variety of plant tissues. It works based on the ability of DNA to bind to silica membrane in the presence of high concentration of chaotropic salts.

Plant DNA molecules bind to the silica-based membrane, and impurities such as proteins and nucleases are removed by thorough washing with Wash Buffer. The DNA is then eluted in sterile, nuclease free water. The isolated DNA is ready to use and should be stored at – 20 °C.

## Intended use

The purified plant DNA is suitable for use in Polymerase Chain Reaction (PCR), real-time PCR, Southern Blotting and Restriction Fragment Length Polymorphism (RFLP) analysis.

## Materials supplied in the Kit

| ***Component*** | ***Quantity*** |
|---|---|
| **Spin Columns** | 100 |
| **Filter Columns** | 100 |
| **Collection tubes** | 200 |
| **Elution tube** | 200 |
| **Elution Buffer** | 30 mL |
| **Binding Buffer 1** | 50 mL |
| **Binding Buffer 2** | 15 mL |
| **Binding Buffer 3** | 30 mL |
| **Wash Buffer 1** | 26 mL |
| **Wash Buffer 2** | 30 mL |
| **RNase A (lyophilized)** | 1 vial |

## Storage instruction

All reagents should be kept at room temperature (RT) except the vial of lyophilized RNase A which must be stored at -20°C upon receipt. Avoid repeated freezing and thawing.


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## Materials required but not supplied

Precision pipettes and disposable filter pipette tips (Nuclease-free)

Vortex and micro-centrifuge (10000 × g)

Sterile 1.5 mL micro-centrifuge tubes (Nuclease-free)

Ethanol 96%-100% (we recommend BioUltra for molecular biology, from Sigma-Aldrich Cat. No. 51976)

## Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

## General remarks

- The instruction must be strictly followed.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

## Reagent preparation

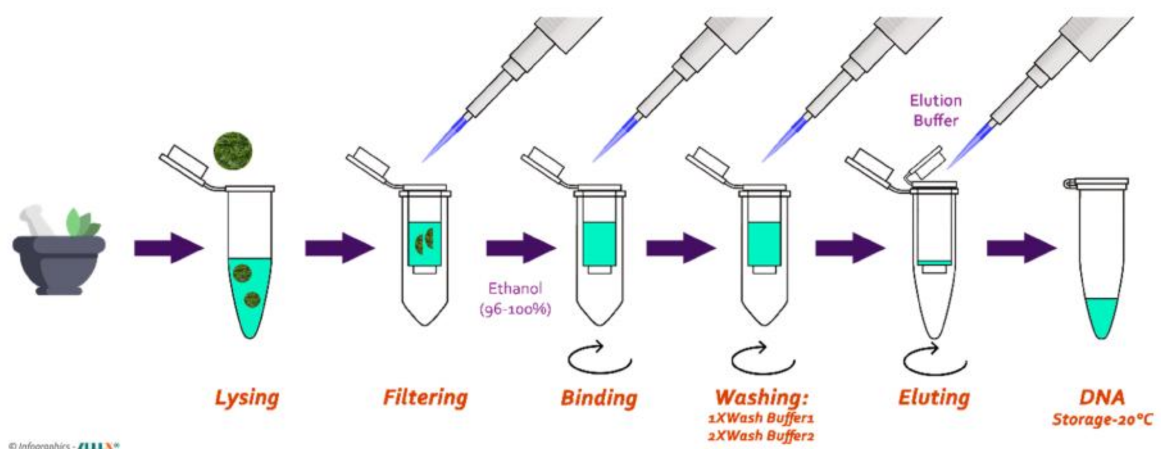
i. **RNase A working solution:** Resuspend the supplied lyophilized vial of RNase A in 840 µL of $ddH_2O$, mix thoroughly until RNase A has been completely dissolved and store at -20 °C.

ii. **Binding Buffer 3 and Wash Buffers 1 and 2:** Add the appropriate amount (according to the bottle label) of molecular biology grade ethanol (96%-100%) [Not provided] to Binding Buffer 3, Wash Buffer 1 and Wash Buffer 2 prior to initial use.

## Assay Procedure

1. Cut and weigh the plant samples (up to 100 mg) and immediately put them inside a mortar with liquid nitrogen.
2. Grind the sample under liquid nitrogen to a fine powder, and transfer to a 1.5 mL microcentrifuge tube (not provided).
3. Add 400 µL of Binding Buffer 1, and 8 µL of RNase A Working Solution, and mix by vigorously vortexing.
4. Incubate at 65°C for 10 minutes. Invert occasionally.
5. Add 130 µL of Binding Buffer 2, mix by vortexing and incubate on ice for 5 minutes.

6. Place a Filter-column in a 2 mL Collection tube and transfer the sample mixture into the Filter-column. Centrifuge at maximal speed for 3 minutes.
7. Transfer the clarified filtrate lysate in a new 1.5 mL microcentrifuge tube (not provided) and add 1.5 valumes of Binding Buffer 3 and mix vigorously by vortexing. (e.g. for 100 µL clarified filtrate lysate add 150 µL Binding Buffer 3)
8. Place a Minispin-column in a 2 mL collection tube and transfer 750 µL of the sample mixture (including any precipitates if present) to the column. Centrifuge at full speed for 1 minute and discard the flow-through.
9. Add the remaining sample mixture from step 7 and centrifuge at full speed for 1 additional minute. Discard the flow-through from the collection tube and place the column back in the same collection tube.
10. Add 400 µL of Wash Buffer 1 and centrifuge at full speed for 30 seconds. Discard the flow-through.
11. Place the Minispin-column in a collection tube and add 650 µL of Wash Buffer 2 and centrifuge at full speed for 30 seconds. Discard the flow-through.
12. Repeat the washing step (step 11) once more using 650 µL of Wash Buffer 2.
13. Centrifuge at full speed for an additional 3 minutes to dry the Minispin-column.
14. Place the Minispin-column into a new, labelled 1.5 microcentrifuge tube (not provided) and pipette 50-100 µL of Elution Buffer (preheated at 65°C) directly onto the membrane (The tip should not touch the membrane). Close the cap and incubate for 3 minutes at RT.
15. Centrifuge at maximum speed for 1 minute to elute DNA and store it at -20 °C.





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