Nexxo-Prep Plant DNA mini

DNA extraction kit, by spin-column system, for isolation of up to 50 µg DNA from plants (leaves, root, fruits, algae, seeds …) or from foods of plant origin (max. 100 mg).

Eluted DNA is ready for further down-stream applications (PCR, sequencing, RFLP analysis, enzymatic digestion, cloning, Southern blot, etc.) or can be stored for future use.

I. Kit components

	5 preps	50 preps	250 preps
Proteinase S	2 ml	2 ml	3 x 2 ml
Binding Solution LSN	2 x 1 ml (ready-to-use)	4 ml (final volume: 15 ml)	2 x 9 ml (final volume: 2 x 30 ml)
Lysis Buffer CF	2 x 2 ml	30 ml	120 ml
Elution Buffer	2 ml	15 ml	60 ml
Wash Solution A	15 ml (ready-to-use)	30 ml (final volume: 60 ml)	80 ml (final volume: 160 ml)
Wash Solution B	15 ml (ready-to-use)	18 ml (final volume: 60 ml)	2 x 45 ml (final volume 2 x 150 ml)
Prefilter	5	50	5 x 50
Spin Filter	5	50	5 x 50
1,5 ml Receiver Tubes	5	50	5 x 50
2,0 ml Receiver Tubes	10	2 x 50	10 x 50
User guide	1	1	1
Art. No.	2032.5	2032.50	2032.250

Required material and equipment not included in this kit

- ddH_2O
- Ethanol >96 %
- Isopropanol >99.7 % (propanol-2 >99.7 %)
- RNase A (10 mg/ ml) : optional
- Reaction tubes (1.5 ml)
- Heating block or water bath (65 °C)
- Microcentrifuge (min 11100 x g)
- Pipettes with corresponding tips
- Disposable gloves

Some components are delivered in concentrated form and have to be diluted appropriately (see chapter « Reagents and buffer solutions preparation », page 2).

II. Storage and stability

All kit components should be stored at room temperature (15-30 °C).

Ethanol and isopropanol are volatile compounds. Keep **Wash Solution A**, **Wash Solution B and Binding Solution LSN** tightly closed.

Note: all kit components are stable for at least 12 months.

Check solutions for absence of precipitates before use. Redisolve precipitates by heating (< 30 °C).

III. Reagents and buffer solutions preparation

1. Kit 5 extractions:

- All components are delivered ready-to-use.

2. Kit 50 extractions:

- Add 11 ml of >99.7 % isopropanol to the **Binding Solution LSN**. Mix by intensive shaking by inverting for 1 min. Store the bottle tightly closed. **Mix briefly, by inverting several times, before use.**
- Add 30 ml of >96 % ethanol to the **Wash Solution A**. Mix completely and store the bottle tightly closed.
- Add 42 ml of >96 % ethanol to the **Wash Solution B**. Mix completely and store the bottle tightly closed.

3. Kit 250 extractions:

- Add 21 ml of >99.7 % isopropanol to each **Binding Solution LSN**. Mix by intensive shaking by inverting for 1 min. Store the bottle tightly closed. **Mix briefly, by inverting several times, before use.**
- Add 80 ml of >96 % ethanol to the **Wash Solution A**. Mix completely and store the bottle tightly closed.
- Add 105 ml of >96 % ethanol to each **Wash Solution B**. Mix completely and store the bottle tightly closed.

IV. Protocol: DNA extraction from plants or foods of plant origin

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter "Reagents and buffer solutions preparation", page 2).
- Ensure that all components are at room temperature and check solutions for absence of precipitates before use. If necessary resuspend precipitates by heating (< 30 °C).
- The last step needs **Elution buffer** heated at 65 °C. Warm up in due time required volume of **Elution buffer** in a 2 ml tube (tube not supplied).
- Mix **Binding Solution LSN** before use (invert several times).

Note: To prevent contamination, use new pipet tip for each pipetting step.

1	**Preparation of the raw material** • Grind the raw material (60 mg) by using a pestle and liquid nitrogen. Other grinding equipment can be used *Note: for raw material with high water content (e.g. fruits), use 120 – 180 mg raw material.*

2	**Lysis** • Transfer the resulting plant powder into a 1.5 ml reaction tube • Add 400 µl of **Lysis Buffer CF** and 20 µl of **Proteinase S** • Vortex briefly • Incubate 30 min. at 65 °C (heating block with constant shacking is recommended)

3	**Filtration of lysis solution** • Put a **Prefilter** into a **2,0 ml Receiver Tube** • Transfer the lysis solution resulting from the step 2 into the **Prefilter** • Centrifuge 1 min. at 11100 x g • Discard the **Prefilter** *Optional: removal of RNA by digestion. Add 40 µl of RNase A (10 mg/ml), vortex briefly, then incubate 5 min. at room temperature.*

Steps 4 to 7 →

4	**DNA adsorption to Spin Filter**
	• Add 200 µl of **Binding Solution LSN** to the resulting solution from previous step • Vortex completely • Put a **Spin Filter** into a new **2,0 ml Receiver Tube** • Transfer the mixture into the **Spin Filter** • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11100 x g • Discard the filtrate and put the **Spin filter** back into the **2.0 ml Receiver tube**

5	**DNA washing, step I**
	• Add 550 µl of **Wash Solution A** • Centrifuge 1 min. at 11000 x g • Discard the filtrate and put the **Spin filter** back into the **2.0 ml Receiver tube**

6	**DNA washing, step II**
	• Add 550 µl of **Wash Solution B** • Centrifuge 1 min. at 11100 x g • Discard the filtrate and put the **Spin filter** back into the **2.0 ml Receiver tube** • Repeat 1 X the washing-centrifugation step • Discard the filtrate and put the **Spin filter** back into the **2.0 ml Receiver tube** • Centrifuge 4 min. at 11100 x g, to remove remaining ethanol

7	**DNA elution**
	• Put the **Spin filter** into a new **1.5 ml Receiver tube** • Add 100 µl of **Elution buffer** (preheated at 65 °C) • Incubate 3 min. at room temperature • Centrifuge 1 min. at 11100 x g *Note: depending on desired yield and concentration, DNA can be eluted with more (max. 200 µl) or less (min. 50 µl) elution buffer.* *Higher yield is reached when eluting twice (2 x 100 µl for instance).*

Eluted DNA is ready for down-stream applications and can directly be used for PCR, sequencing, RFLP analysis, enzymatic digestion, cloning, Southern blot, etc., or can be stored at 4 °C for several weeks.

For long-time storage, store eluted DNA at -20 °C.

*Note: **Elution buffer** contains no EDTA. For enhanced stability during long-time storage, it is recommended to store DNA in a Tris-EDTA buffer.*

To increase the yield of an ethanol precipitation, it is better to use air drying rather than vacuum treatment.

DNA storage at -20 °C is subject to shearing forces. Avoid multiple freeze/thaw cycles.