Nexxo-Prep duo, Gel Extraction & PCR Clean-Up

DNA extraction, by spin-column system, for PCR products, restriction digestion or cDNA synthesis purification and for DNA purification from up to 300 mg agarose gels (TAE, TBE).

The purification from agarose gels can be achieved in under 20 minutes, with a yield of up to 90 %. (DNA size: 80 bp – 30 kb)

PCR products, restriction digestion or cDNA synthesis purification can be achieved in approx. 5 minutes, with an effective removal of primers, enzymes, unincorporated nucleotides, dyes or other impurities, with a yield of up to 95 %. (DNA size: 80 bp – 30 kb, max. 100 µl)

Eluted DNA is ready for down-stream applications and can directly be used for sequencing, cloning, ligation, enzymatic digestion, hybridization, transformation, labelling, amplification, etc., or can be stored for future use.

I. Kit components

	10 preps	50 preps	250 preps
Elution Buffer	2 ml	2 x 2 ml	15 ml
Binding Amplifier	2 ml (final volume: 10 ml)	6 ml (final volume: 30 ml)	30 ml (final volume: 150 ml)
Wash Solution S	15 ml (ready to use)	18 ml (final volume: 60 ml)	2 x 45 ml (final volume: 2 x 150 ml)
Binding Solution S1	4 ml (final volume: 11 ml)	12 ml (final volume: 32 ml)	63 ml (final volume: 163 ml)
Gelsol	12 ml	60 ml	2 x 140 ml
Spin Filter	10	50	5 x 50
1,5 ml Receiver Tubes	10	50	5 x 50
2,0 ml Receiver Tubes	10	50	5 x 50
User guide	1	1	1
Art. No.	2038.10	2038.50	2038.250

Required material and equipment not included in this kit

- Isopropanol >99.7 % (propanol-2 >99.7 %)
- Ethanol >96 %
- Reaction tubes (1.5 ml and 2.0 ml))
- Heating block or water bath (65 °C)
- Microcentrifuge (min 11100 x g ; approx. 11000 rpm)
- 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 S**, **Binding Amplifier and Binding Solution S1** 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 10 extractions:

- Add 7 ml of >99.7 % isopropanol to the **Binding Solution S1**
- Add 8 ml of >99.7 % isopropanol to the **Binding Amplifier**

*Note: in the 10 extractions kit the **Wash Solution S** is delivered ready-to-use.*

2. Kit 50 extractions:

- Add 20 ml of >99.7 % isopropanol to the **Binding Solution S1**
- Add 24 ml of >99.7 % isopropanol to the **Binding Amplifier**
- Add 42 ml of >96 % ethanol to the **Wash Solution S**

3. Kit 250 extractions:

- Add 100 ml of >99.7 % isopropanol to the **Binding Solution S1**
- Add 120 ml of >99.7 % isopropanol to the **Binding Amplifier**
- Add 105 ml of >96 % ethanol to each **Wash Solution S**

IV. Protocol 1: purification of PCR, restriction digestions or cDNA synthesis products

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).

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

Depending on sample characteristics/volume, start with step 1a, 1b or 1c

1a	**DNA adsorption to the Spin filter** (sample volume: max. 50 µl)
	• Add 250 µl of **Binding Solution S1** to the sample (max. 50 µl) *Note: if the sample contains mineral oil (e.g. PCR samples …), start with step 1b instead of step 1a.* • Mix completely by pipetting or vortex • Put a **Spin Filter** into a **2.0 ml Receiver tube** • Transfer the whole sample mixture, containing **Binding Solution S1,** into the **Spin Filter** • Centrifuge 2 min. at 11000 x g • Proceed with step 2 « **DNA elution** »

1b	**DNA adsorption to the Spin filter** (sample volume: 50 - 100 µl)
	• Add 500 µl of **Binding Solution S1** to the sample (50 - 100 µl) • Mix completely by pipetting or vortex • Put a **Spin Filter** into a **2.0 ml Receiver tube** • Transfer the whole sample mixture, containing **Binding Solution S1,** into the **Spin Filter** • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin Filter** back into the **2.0 ml Receiver tube** • Centrifuge 3 min. at 11000 x g • Proceed with step 2 « **DNA elution** »

Steps 1c to 2 →

1c	**DNA adsorption to the Spin filter** (sample volume: max. 200 µl)
	• Add 1000 µl of **Binding Solution S1** to the sample (max. 200 µl) *Note: if the sample contains mineral oil (e.g. PCR samples …), increase volume of **Binding Solution S1** by 500 µl.* • Mix completely by pipetting or vortex • Put a **Spin Filter** into a **2.0 ml Receiver tube** • Transfer approx. half of the sample mixture, containing the **Binding Solution S1,** into the **Spin Filter** • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin Filter** back into the **2.0 ml Receiver tube** • Transfer the remaining half of the sample mixture, containing the **Binding Solution S1,** into the **Spin Filter** • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin Filter** back into the **2.0 ml Receiver tube** • Centrifuge 3 min. at 11000 x g • Proceed with step 2 « **DNA elution** »

2	**DNA elution**
	• Put the **Spin filter** into a new **1.5 ml Receiver tube** • Add, at least, 10 µl of **Elution Buffer** (or ddH_2O, or Tris buffer) into the center of the filter • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g *Note: increasing the incubation time by 5 min. enhances slightly the yield.*

Eluted DNA is ready for down-stream applications and can directly be used for sequencing, cloning, ligation, enzymatic digestion, hybridization, transformation, labelling, etc., or can be stored at 4 °C for several weeks.

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

Note: this kit has been calculated for samples up to 100 µl. The protocol for 200 µl samples (1c) reduces the total number of purifications.

V. Protocol 2: DNA extraction and purification from up to 300 mg agarose gel slice (TAE, TBE)

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter "Reagents and buffer solutions preparation", page 2).
- Preheat a heating block (50 °C), for the agarose gel solubilization step.
- 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).

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

1	**Agarose gel solubilization** • Excise an agarose gel slice (up to 300 mg) • Transfer the slice into a 1.5 ml or 2.0 ml reaction tube • Add the required volume(*) of **Gelsol** (*) Gel slice of up to 150 mg: 500 µl of **Gelsol** 150 – 300 mg gel slice: 1 ml of **Gelsol** • Incubate 10 min. at 50 °C, until complete dissolution of the gel slice

2	**DNA adsorption to the Spin filter** • Add the required volume(*) of **Binding Amplifier** (*) Gel slice of up to 150 mg: 250 µl of **Binding Amplifier** 150 – 300 mg: 500 µl of **Binding Amplifier** • Mix by pipetting or vortex • Put a **Spin Filter** into a **2.0 ml Receiver tube** • Transfer 800 µl of the sample mixture, containing **Binding Amplifier,** into the **Spin Filter** • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin Filter** back into the **2.0 ml Receiver tube** *Note: if there is some remaining mixture, containing **Binding Amplifier**. Reiterate the centrifugation with this remaining mixture.*

Steps 3 to 4 →

	DNA washing
3	• Add 500 µl of **Wash Solution S** to the **Spin Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **Spin filter** back into the **2.0 ml Receiver tube** • Repeat 1 X the washing-centrifugation step • Discard the flow-through and put the **Spin filter** back into the **2.0 ml Receiver tube** • Centrifuge 4 min. at 11000 x g to remove remaining ethanol from the **Spin Filter**

	DNA elution
4	• Put the **Spin filter** into a new **1.5 ml Receiver tube** • Add 20 - 50 µl of **Elution Buffer** (or ddH_2O, or Tris buffer) into the center of the filter • Incubate 5 min. at room temperature • Centrifuge 1 min. at 11000 x g *Note: increasing the incubation time by 5 min. enhances slightly the yield.*

Eluted DNA is ready for down-stream applications and can directly be used for sequencing, cloning, ligation, enzymatic digestion, hybridization, transformation, labelling, amplification etc., or can be stored at 4 °C for several weeks.

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