Nexxo-Prep Tissue DNA mini

DNA extraction kit, by spin-column system, for isolation of up to 50 µg DNA from tissue (max. 40 mg), paraffin embedded tissue, rodent tail (max. 1.2 cm), insects, animal origin food samples, eukaryotic cells (max. 1.10^6 cells) or swabs.

Eluted DNA is ready for down-stream applications and can directly be used for PCR, sequencing, enzymatic digestion, Southern blot, etc., or can be stored for future use.

I. Kit components

	5 preps	50 preps	250 preps
Elution Buffer	2 ml	30 ml	120 ml
Proteinase S	2 ml	2 x 2 ml	6 x 2 ml
Lysis Buffer RS	2 x 2 ml	30 ml	120 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)
Wash Solution S	15 ml (ready to use)	18 ml (final volume: 60 ml)	2 x 45 ml (final volume: 2 x 150 ml)
Spin Filter	5	50	5 x 50
1,5 ml Receiver Tubes	5	50	5 x 50
2,0 ml Receiver Tubes	5	50	5 x 50
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Some components are delivered in concentrated form and have to be diluted appropriately (see chapter « Reagents and buffer solutions preparation », page 2).

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
- Octane: optional (deparaffination)
- PBS (FFPE protocol)
- DTT 1M (FFPE protocol)
- Reaction tubes (1.5 ml or 2.0 ml)
- Heating block or water bath (52 °C)
- Microcentrifuge (13400 x g)
- Pipettes with corresponding tips
- Disposable gloves

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 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 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 42 ml of >96 % ethanol to the **Wash Solution S**. 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 105 ml of >96 % ethanol to each **Wash Solution S**. Mix completely and store the bottle tightly closed.

IV. Protocol 1: DNA extraction from tissue (0.5 - 40 mg), rodent tail (max. 1.2 cm), biopsies, insects or animal origin food samples

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 52 °C. Warm up in due time required volume of **Elution buffer** (50-200 µl).
- Mix **Binding Solution LSN** before use (invert several times).
- For liver tissue, it is recommended not to use more than 20 mg samples.

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

1	**Preparation of the raw material** • Put the raw material sample into a 1.5 ml reaction tube (not supplied) Preliminary mechanical grinding (mortar/pestle, scissors…) improves yields. • Add 400 µl of **Lysis Buffer RS** and 40 µl of **Proteinase S** • Vortex exhaustively • Incubate at 52 °C, with constant shacking, until complete lysis. Vortex several times if necessary *Optional: an overnight incubation is feasible for particularly resistant raw materials (e.g. rodent tail)* • Centrifuge 2 min. at 11000 x g, to spin down non-lysed material • Transfer the supernatant to a new 1.5 ml reaction tube (tube not supplied) *Optional: removal of RNA by digestion. Add 40 µl of RNase A (10 mg/ml), vortex briefly, then incubate 5 min. at room temperature.*

2	**DNA adsorption to Spin Filter** • Add 200 µl of **Binding Solution LSN** • Vortex 10 sec. • Insert a **Spin Filter** into a **2.0 ml Receiver tube** • Transfer the suspension into the **Spin Filter** • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin filter** back into the **2.0 ml Receiver tube**

Steps 3 to 4 →

3	**DNA washing** • Add 550 µl of **Wash Solution S** • 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 max. speed to remove remaining ethanol

4	**DNA elution** • Insert the **Spin filter** into a new **1.5 ml Receiver tube** • Add 200 µl of **Elution buffer** (preheated at 52 °C) • Incubate 3 min. at room temperature • Centrifuge 1 min. at 11000 x g • Eluted DNA is ready-to-use *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, enzymatic digestion, 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.

Note: elution can also be carried out with ddH_2O.

V. Protocol 2: DNA extraction from paraffin embedded tissue (FFPE)

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 52 °C. Warm up in due time required volume of **Elution buffer** (50-200 µl).
- Mix **Binding Solution LSN** before use (invert several times).
- Prepare PBS (not supplied) and 1 M DTT (not supplied)

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

1	**Dewaxing** • Transfer the sample into a 1.5 ml reaction tube (not supplied) • Add 1 ml of octane • Vortex gently until paraffin is dissolved and tissue looks transparent (paraffin remains white) • Centrifuge 2 min. at 11000 x g • Remove delicately the supernatant (retain only the pellet) *Note: if it remains some paraffin, centrifuge again 2 min. at 11000 x g and remove delicately the supernatant.* • Add 0.5 ml of ethanol >96 % to the pellet • Mix completely • Centrifuge briefly • Remove ethanol with a pipette • Incubate the open tube at 52 °C to remove the remaining ethanol

2	**Formalin elimination** • Add 1 ml of PBS supplemented with 2 µl of 1 M DTT • Incubate 20 min. at 99 °C under continuous shacking • Centrifuge 1 min. at 11000 x g • Remove delicately the supernatant (retain only the pellet) • Add 1 ml of PBS • Vortex gently • Centrifuge 1 min. at 11000 x g • Remove delicately the supernatant (retain only the pellet)

Step 3 →

3	**Cell lysis** • Add 400 µl of **Lysis Buffer RS**, 40 µl of **Proteinase S** and 0.4 µl of 1 M DTT *Note: preliminary mechanical grinding improves yields.* • Vortex exhaustively • Incubate at 52 °C, with constant shacking, until complete lysis (min. 2 hours). • Centrifuge 2 min. at 11000 x g, to spin down non-lysed material • Transfer the supernatant to a new 1.5 ml reaction tube (tube not supplied) *Optional: removal of RNA by digestion. Add 40 µl of RNase A (10 mg/ml), vortex briefly, then incubate 5 min. at room temperature.*

Proceed with steep 2 (“DNA adsorption to Spin Filter “) of protocol 1, page 3.

Note:

- *To extract DNA from a paraffin embedded tissue which is not formalin fixed, omit step 2 (“Formalin elimination”) and pass directly from step 1 (“Dewaxing”) to step 3 (“Cell lysis”).*
- *To extract DNA from formalin fixed tissue which is not paraffin embedded, omit step 1 (“Dewaxing”) and start directly with step 2 (“Formalin elimination”) by placing the sample in a 1.5 ml reaction tube (not supplied).*

VI. Protocol 3a: DNA extraction from eukaryotic cells (10 – 10^6)

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 52 °C. Warm up in due time required volume of **Elution buffer** (50-200 µl).
- Mix **Binding Solution LSN** before use (invert several times).

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

1	**Cell harvesting** a) <u>From a cell suspension</u> • Centrifuge 5 min at 300 x g, the cell culture containing up to 1.10^6 cells • Discard carefully the supernatant and the whole culture medium (do not disturb the pellet) • Wash the pellet (with PBS for instance) b) <u>From a cell monolayer</u> • Detach the cells by trypsinization • Transfer the cells into a 50 ml centrifuge tube • Centrifuge 5 min at 300 x g • Discard carefully the supernatant and the whole culture medium (do not disturb the pellet) • Wash the pellet (with PBS for instance)

2	**Cell lysis** • Add 400 µl of **Lysis Buffer RS** and 40 µl of **Proteinase S** to the washed cell pellet • Vortex exhaustively • Transfer the sample mixture to a 1.5 ml reaction tube (not supplied) • Incubate at 52 °C, with constant shacking, until complete lysis. Vortex several times if necessary • Centrifuge 2 min. at 11000 x g to spin down non-lysed material • Transfer the supernatant to a new 1.5 ml reaction tube (tube not supplied) *Optional: removal of RNA by digestion. Add 40 µl of RNase A (10 mg/ml), vortex briefly, then incubate 5 min. at room temperature.*

Proceed with steep 2 ("DNA adsorption to Spin Filter ") of protocol 1, page 3.

VII. Protocol 3b: DNA extraction from apoptotic eukaryotic cells (10 – 10^6)

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 52 °C. Warm up in due time required volume of **Elution buffer** (50-200 µl).
- Mix **Binding Solution LSN** before use (invert several times).

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

1	**Cell harvesting** a) <u>From a cell suspension</u> • Centrifuge 5 min at 300 x g, the cell culture containing up to 1.10^6 cells • Discard carefully the supernatant and the whole culture medium (do not disturb the pellet) • Wash the pellet (with PBS for instance) b) <u>From a cell monolayer</u> • Detach the cells by trypsinization • Transfer the cells into a 50 ml centrifuge tube • Centrifuge 5 min at 300 x g • Discard carefully the supernatant and the whole culture medium (do not disturb the pellet) • Wash the pellet (with PBS for instance)

2	**Cell lysis** • Add 400 µl of **Lysis Buffer RS** and 40 µl of **Proteinase S** to the washed cell pellet • Vortex exhaustively • Transfer the sample mixture to a 1.5 ml reaction tube (not supplied) • Incubate 15 min. at 52 °C, with constant shacking, until complete lysis. Vortex several times if necessary • Centrifuge 2 min. at 11000 x g to spin down non-lysed material • Transfer the supernatant to a new 1.5 ml reaction tube (tube not supplied) *Optional: removal of RNA by digestion. Add 40 µl RNase A (10 mg/ml), vortex briefly, then incubate 5 min. at room temperature.*

Proceed with steep 2 ("DNA adsorption to Spin Filter ") of protocol 1, page 3.

VIII. Protocol 4a: DNA extraction from swab

*Note: this protocol requires a larger amount of **Lysis Buffer RS and Binding Solution LSN.** Using this protocol reduce the total number of extractions.*

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).

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- The last step needs **Elution buffer** heated at 52 °C. Warm up in due time required volume of **Elution buffer** (50-200 µl).
- The step "Sample preparation" requires to heat the sample at 65 °C. Preheat a heating block or water bath.
- Mix **Binding Solution LSN** before use (invert several times).

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

1	**Sample preparation** • Transfer 600 µl of **Lysis Buffer RS** and 40 µl of **Proteinase S** into a 1.5 ml tube • Put the swab into the 1.5 ml tube *Note: if the swab was in a transport media. Centrifuge the swab 1 min. at max. speed. Discard the supernatant (retain the pellet and the swab). Resuspend the pellet by adding the **Lysis Buffer RS** and the **Proteinase S** to the tube with the swab inside.* • Incubate 15 min. at 65 °C, with continuous shaking • Squeeze carefully the swab on the inner tube wall, then discard the swab

2	**DNA adsorption to Spin Filter** • Add 300 µl of **Binding Solution LSN** • Mix completely • Insert a **Spin Filter** into a **2.0 ml Receiver tube** • Transfer the suspension into the **Spin Filter** • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin filter** back into the **2.0 ml Receiver tube**

Proceed with steep 3 ("DNA washing ") of protocol 1, page 4.

IX. Protocol 4b: DNA extraction from swab by rinsing

*Note: this protocol requires a larger amount of **Lysis Buffer RS and Binding Solution LSN.** Using this protocol reduce the total number of extractions.*

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).
- Mix **Binding Solution LSN** before use (invert several times).
- The last step needs **Elution buffer** heated at 52 °C. Warm up in due time required volume of **Elution buffer** (50-200 µl).
- The step "Sample preparation" requires to heat the sample at 65 °C. Preheat a heating block or water bath.
- The step "Sample preparation" requires to cool the **Lysis Buffer RS** at 4 °C.

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

	Sample preparation
1	• Rinse the swab with 600 µl of **Lysis Buffer RS** cooled at 4 °C, into a tube (not supplied) • Squeeze carefully the swab on the inner tube wall, then discard the swab • Transfer the solution into a **1.5 ml Receiver tube** (supplied) • Add 40 µl of **Proteinase S** • Incubate 15 min. at 65 °C, with shaking

	DNA adsorption to Spin Filter
2	• Add 300 µl of **Binding Solution LSN** • Mix completely • Insert a **Spin Filter** into a **2.0 ml Receiver tube** • Transfer the suspension into the **Spin Filter** • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin filter** back into the **2.0 ml Receiver tube**

Proceed with steep 3 ("DNA washing ") of protocol 1, page 4.