Nexxo-Prep Blood Genomic DNA mini

DNA extraction, by spin-column system, for the isolation of up to 10 µg genomic DNA from mammalian blood (max. 200 µl), non-mammalian blood (max. 25 µl), buffy coat (max. 30 µl) or bone marrow (max. 20 µl).

Eluted DNA is ready for down-stream applications and can directly be used for PCR, cloning, etc., or can be stored for future use.

Note: this kit is compatible with EDTA or citrate treated blood samples, but is not appropriated for heparin stabilized samples.

I. Kit components

	5 preps	50 preps	250 preps
Protéinase S (Proteinase S)	2 ml (ready-to-use)	2 ml (ready-to-use)	3 x 2 ml (ready-to-use)
Tampon d'élution (Elution Buffer)	2 ml	15 ml	60 ml
Solution de fixation BM (Binding Solution BM)	2 x 1 ml (ready-to-use)	4 ml (final volume: 16 ml)	2 x 8 ml (final volume: 2 x 32 ml)
Tampon de lyse GLT (Lysis Buffer GLT)	2 ml	15 ml	60 ml
Solution de lavage L1 (Wash Solution L1)	15 ml (ready-to-use)	30 ml (final volume: 60 ml)	80 ml (final volume: 160 ml)
Solution de lavage S (Wash Solution S)	15 ml (ready-to-use)	2 x 18 ml (final volume: 2 x 60 ml)	3 x 45 ml (final volume: 3 x 150 ml)
Kit filtres de centrifugation GS (Spin Filter Set GS)	5	50	5 x 50
Tubes receveurs GS (Receiver Tubes GS)	15	3 x 50	15 x 50
Tubes receveurs 1,5 ml (1,5 ml Receiver Tubes)	10	2 x 50	10 x 50
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Required material and equipment not included in this kit

- ddH_2O
- Ethanol >96 %
- Isopropanol >99.7 % (propanol-2 >99.7 %)
- 1X PBS: optional
- Reaction tubes (1.5 ml or 2.0 ml)
- Heating block or water bath (56 °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 L1**, **Wash Solution S and Binding Solution BM** tightly closed.

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

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

III. Reagents and buffer solutions preparation

1. Kit 5 extractions:

- All components are delivered ready-to-use

2. Kit 50 extractions:

- Add 12 ml of >99.7 % isopropanol to the **Binding Solution BM**. 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 L1**. Mix thoroughly and store the bottle tightly closed.
- Add 42 ml of >96 % ethanol to each **Wash Solution S**. Mix thoroughly and store the bottle tightly closed.

3. Kit 250 extractions:

- Add 24 ml of >99.7 % isopropanol to each **Binding Solution BM**. 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 L1**. Mix thoroughly and store the bottle tightly closed.
- Add 105 ml of >96 % ethanol to each **Wash Solution S**. Mix thoroughly and store the bottle tightly closed.

IV. Protocol 1: DNA extraction from 1 – 200 µl mammalian blood (e.g. human) or 1 - 30 µl buffy coat

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
- Check solutions for absence of precipitates before use. If necessary resuspend precipitates by heating (< 30 °C).
- Preheat a heating block (56 °C) for the lysis step
- The last step needs **Elution buffer** heated at 56 °C. Warm up in due time required volume of **Elution buffer** in a 2 ml tube (tube not supplied).
- Mix briefly the **Binding Solution BM** before use (invert several times).

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

1	**Cell lysis** • Transfer 1 – 200 µl of whole blood (or 1 – 30 µl of buffy coat) into a 1.5 ml reaction tube *Note: for sample volumes under 200 µl, adjust the volume at 200 µl with 1X PBS or ddH_2O* • Add 200 µl of **Lysis Buffer GLT** • Vortex 15 sec. • Incubate 3 min. under continuous shacking at 56 °C • Add 20 µl of **Proteinase S** • Vortex briefly • Incubate 5 min. under continuous shacking at 56 °C

2	**DNA adsorption to Spin filter GS** • Add 200 µl of **Binding Solution BM** (invert several times before use) • Vortex effectively 15 sec. • Transfer the mixture into the filter of a **Spin Filter Set GS** (filter inserted in its receiver tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and the receiver tube • Put the **Spin Filter GS** into a new **Receiver Tube GS**

Steps 3 to 6 →

3	**DNA washing, step I** • Add 500 µl of **Wash Solution L1** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and the receiver tube • Put the **Spin Filter GS** into a new **Receiver Tube GS**

4	**DNA washing, step II** • Add 700 µl of **Wash Solution S** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and the receiver tube • Put the **Spin Filter GS** into a new **Receiver Tube GS**

5	**DNA washing, step III** • Add 700 µl of **Wash Solution S** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **Spin filter GS** back into the **Receiver tube GS** • Centrifuge 4 min. at max. speed to remove remaining ethanol

6	**DNA elution** • Insert the **Spin Filter GS** into a new **1.5 ml Receiver Tube** • Add 200 µl of **Elution buffer** (preheated at 56 °C) • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the **Spin Filter GS** and store the ready-to-use DNA at 4 °C *Note: depending on desired yield and concentration, DNA can be eluted with more (max. 200 µl) or less (min. 30 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, SNP analysis, HLA testing, enzymatic digestion, cloning, 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.*

V. Protocol 2: DNA extraction from 1 – 25 µl non mammalian blood

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
- Check solutions for absence of precipitates before use. If necessary resuspend precipitates by heating (< 30 °C).
- Preheat a heating block (56 °C) for the lysis step
- The last step needs **Elution buffer** heated at 56 °C. Warm up in due time required volume of **Elution buffer** in a 2 ml tube (tube not supplied).
- Prepare a 1X PBS solution
- Mix briefly the **Binding Solution BM** before use (invert several times).

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

	Cell lysis
1	• Transfer 1 – 25 µl of whole blood into a 1.5 ml reaction tube • Adjust with 1X PBS at 200 µl • Add 200 µl of **Lysis Buffer GLT** • Vortex 15 sec. • Incubate 3 min. under continuous shacking at 56 °C • Add 20 µl of **Proteinase S** • Vortex briefly • Incubate 5 min. under continuous shacking at 56 °C

	DNA adsorption to Spin filter GS
2	• Add 200 µl of **Binding Solution BM** (invert several times before use) • Vortex effectively 15 sec. • Transfer the mixture into the filter of a **Spin Filter Set GS** (filter inserted in its receiver tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and the receiver tube • Put the **Spin Filter GS** into a new **Receiver Tube GS**

Steps 3 to 6 →

3	**DNA washing, step I** • Add 500 µl of **Wash Solution L1** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and the receiver tube • Put the **Spin Filter GS** into a new **Receiver Tube GS**

4	**DNA washing, step II** • Add 700 µl of **Wash Solution S** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and the receiver tube • Put the **Spin Filter GS** into a new **Receiver Tube GS**

5	**DNA washing, step III** • Add 700 µl of **Wash Solution S** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **Spin filter GS** back into the **Receiver tube GS** • Centrifuge 4 min. at max. speed to remove remaining ethanol

6	**DNA elution** • Insert the **Spin Filter GS** into a new **1.5 ml Receiver Tube** • Add 200 µl of **Elution buffer** (preheated at 56 °C) • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the **Spin Filter GS** and store the ready-to-use DNA at 4 °C *Note: depending on desired yield and concentration, DNA can be eluted with more (max. 200 µl) or less (min. 30 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, SNP analysis, enzymatic digestion, cloning, 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.*

VI. Protocol 3: DNA extraction from bone marrow 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.
- Check solutions for absence of precipitates before use. If necessary resuspend precipitates by heating (< 30 °C).
- Preheat a heating block (56 °C) for the lysis step.
- The last step needs **Elution buffer** heated at 56 °C. Warm up in due time required volume of **Elution buffer** in a 2 ml tube (tube not supplied).
- Prepare a 1X PBS solution
- Mix briefly the **Binding Solution BM** before use (invert several times).

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

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

1a	**Preparation of dry bone marrow**
	• Humidify the dry matter with a drop of PBS • Transfer 180 µl of PBS in a 1.5 ml reaction tube (not supplied) • Scrape the humidified matter into the 1.5 ml reaction tube *Note: use the edge of clean slide for scrapping the matter* • Suspend the matter by pipetting up and down • Proceed with step 2 « **Cell lysis** »

1b	**Preparation of fresh bone marrow**
	• Transfer 1 – 20 µl of bone marrow into a 1.5 ml reaction tube (not supplied) • Proceed with step 2 « **Cell lysis** »

2	**Cell lysis**
	• Adjust with 1X PBS at 200 µl • Add 200 µl of **Lysis Buffer GLT** • Vortex 15 sec. • Incubate 3 min. under continuous shacking at 56 °C • Add 20 µl of **Proteinase S** • Vortex briefly (10 sec.) • Incubate 5 min. under continuous shacking at 56 °C

Steps 3 to 7 →

3	**DNA adsorption to Spin filter GS** • Add 200 µl of **Binding Solution BM** (invert several times before use) • Vortex effectively 15 sec. • Transfer the mixture into the filter of a **Spin Filter Set GS** (filter inserted in its receiver tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and the receiver tube • Put the **Spin Filter GS** into a new **Receiver Tube GS**

4	**DNA washing, step I** • Add 500 µl of **Wash Solution L1** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and the receiver tube • Put the **Spin Filter GS** into a new **Receiver Tube GS**

5	**DNA washing, step II** • Add 700 µl of **Wash Solution S** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and the receiver tube • Put the **Spin Filter GS** into a new **Receiver Tube GS**

6	**DNA washing, step III** • Add 700 µl of **Wash Solution S** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **Spin filter GS** back into the **Receiver tube GS** • Centrifuge 4 min. at max. speed, to remove remaining ethanol

7	**DNA elution** • Insert the **Spin Filter GS** into a new **1.5 ml Receiver Tube** • Add 200 µl of **Elution buffer** (preheated at 56 °C) • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the **Spin Filter GS** and store the ready-to-use DNA at 4 °C *Note: depending on desired yield and concentration, DNA can be eluted with more (max. 200 µl) or less (min. 30 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, SNP analysis, HLA testing, enzymatic digestion, cloning, 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.*