Nexxo-Prep Bacteria DNA mini

DNA extraction, by spin-column system, for extraction of bacterial DNA from tissue (1-10 mg), food samples (25 g), bacteria pellets (up to 1.10^9 cells), paraffin embedded tissue (FFPE), urine and water samples, or paper points.

Eluted DNA is ready for further down-stream applications and can be directly used for PCR, real-time PCR, RFLP-analysis or can be stored for future use.

Note: this kit use a poly A carrier RNA (100 up to 1000 bases) and is not appropriated if DNA is to be used in gel electrophoresis or capillary electrophoresis (amount poly A extracted > amount DNA extracted). Directly after isolation, DNA is single-stranded and staining with an intercalating agent is only partially effective.

I. Kit components

	5 preps	50 preps	250 preps
Elution Buffer	2 ml	30 ml	120 ml
Binding Solution XT	3 x 1 ml (ready-to-use)	9 ml (final volume: 30 ml)	36 ml (final volume: 120 ml)
Resuspension Buffer RSB	2 x 2 ml	30 ml	150 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)	60 ml (final volume: 200 ml)
Spin Filter Set GS	5	50	5 x 50
Receiver Tubes GS	5	50	5 x 50
1,5 ml Receiver Tubes	5	50	5 x 50
Extraction Tubes	5	50	5 x 50
User guide	1	1	1
Art. No.	2033.5	2033.50	2033.250

Required material and equipment not included in this kit

- ddH_2O
- Ethanol >96 %
- Isopropanol >99.7 %
- Octane: optional (deparaffination)
- Reaction tubes (1.5 ml)
- Heating block or water bath (95 °C)
- Microcentrifuge (11000 x g)
- Centrifuge for 15 - 50 ml tubes: optional
- 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 XT** tightly closed.

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

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

III. Reagents and buffer solutions preparation

1. Kit 5 extractions:

*Note: in the 5 extractions kit, **Binding Solution XT, Wash Solution A** and **Wash Solution B** are delivered ready-to-use.*

2. Kit 50 extractions:

- Add 21 ml of >99.7 % isopropanol to the **Binding Solution XT**. 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 briefly and store the bottle tightly closed.
- Add 42 ml of >96 % ethanol to the **Wash Solution B**. Mix briefly and store the bottle tightly closed.

3. Kit 250 extractions:

- Add 84 ml of >99.7 % isopropanol to the **Binding Solution XT**. 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 briefly and store the bottle tightly closed.
- Add 140 ml of >96 % ethanol to the **Wash Solution B**. Mix briefly and store the bottle tightly closed.

IV. Protocol 1: bacterial DNA extraction from swab

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter “Reagents and buffer solutions preparation”, page 2).
- Check solutions for absence of precipitates before use. If necessary resuspend precipitates by heating (< 30 °C).
- Preheat a heating block or water bath (37 °C, 65 °C and 95 °C will be required).
- 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).

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

Depending on bacteria type, start with step 1a (Gram +) or step 1b (Gram -)

1a	**Lysis of Gram positive bacteria**
	• Put the swab into the **Extraction tube** • Add 400 µl of **Resuspension Buffer RSB** • Mix by stirring the swab • Cut the swab and close the **Extraction tube** • Incubate in a heating block, 10 min. at 37 °C • Incubate in a heating block, 10 min. at 65 °C (if the transition from 37 °C to 65 °C is very time-consuming, increase incubation time by 2 min.) *Note: if the transition from 37 °C to 65 °C is longer than 7 min., increase incubation time by more than 2 min.* • Squeeze the swab on the inner tube wall and remove it from the **Extraction tube** • Incubate **Extraction tube** in a heating block, 5 - 10 min. at 95 °C *Note: incubation under continuous shaking increase lysis efficiency* • Proceed with step 2 **« DNA adsorption to Spin filter GS »**

1b	**Lysis of Gram negative bacteria**
	• Put the swab into the **Extraction tube** • Add 400 µl of **Resuspension Buffer RSB** • Mix by stirring the swab • Cut the swab and close the **Extraction tube** • Incubate in a heating block, 10 min. at 65 °C • Squeeze the swab on the inner tube wall and remove it from the **Extraction tube** • Incubate **Extraction tube** in a heating block, 5 - 10 min. at 95 °C *Note: incubation under continuous shaking increase lysis efficiency* • Proceed with step 2 **« DNA adsorption to Spin filter GS »**

Steps 2 to 5 →

2	**DNA adsorption to Spin filter GS**
	• Add 400 µl of **Binding Solution XT** • Vortex briefly • Transfer the entire sample mixture into a **Spin Filter Set GS** (filter with tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin filter GS** back into the **Receiver tube GS**

3	**DNA washing, step I**
	• Add 500 µl of **Wash Solution A** • 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 600 µl of **Wash Solution B** • 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 in order to remove remaining ethanol

5	**Elution of bacterial DNA**
	• Put the **Spin filter GS** into a new **1.5 ml Receiver tube** • Add 100 – 200 µl of **Elution buffer** (preheated at 65 °C) • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Store the eluted bacterial DNA at +4°C *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 further down-stream applications and can be directly used for PCR, real-time PCR, RFLP-analysis or stored at 4 °C for several weeks.

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

Warning !: Nexxo-Prep Bacteria DNA mini kit use a poly A **carrier RNA** and is not appropriated if DNA is directly used in gel electrophoresis or capillary electrophoresis.

Note: elution can also be carried out with ddH_2O.

V. Protocol 2: DNA extraction from bacteria pellets

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter "Reagents and buffer solutions preparation", page 2).
- Check solutions for absence of precipitates before use. If necessary resuspend precipitates by heating (< 30 °C).
- Preheat a heating block or water bath (37 °C, 65 °C and 95 °C will be required).
- 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).

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

Depending on bacteria type, start with step 1a (Gram +) or step 1b (Gram -)

1a	**Lysis of Gram positive bacteria**
	• Add 400 µl of **Resuspension Buffer RSB** to the pellet • Resuspend the pellet by pipetting up and down • Transfer the entire suspension in an **Extraction tube** • Vortex briefly • Incubate in a heating block, 10 min. at 37 °C • Incubate in a heating block, 10 min. at 65 °C (if the transition from 37 °C to 65 °C is very time-consuming, increase incubation time by 2 min.) *Note: if the transition from 37 °C to 65 °C is longer than 7 min., increase incubation time by more than 2 min.* • Incubate **Extraction tube** in a heating block, 5 - 10 min. at 95 °C *Note: incubation under continuous shaking increase lysis efficiency* • Proceed with step 2 **« DNA adsorption to Spin filter GS »**

1b	**Lysis of Gram negative bacteria**
	• Add 400 µl of **Resuspension Buffer RSB** to the pellet • Resuspend the pellet by pipetting up and down • Transfer the entire suspension in an **Extraction tube** • Vortex briefly • Incubate in a heating block, 10 min. at 65 °C • Incubate **Extraction tube** in a heating block, 5 - 10 min. at 95 °C *Note: incubation under continuous shaking increase lysis efficiency* • Proceed with step 2 **« DNA adsorption to Spin filter GS »**

Steps 2 to 5 →

2	**DNA adsorption to Spin filter GS**
	• Add 400 µl of **Binding Solution XT** • Vortex briefly • Transfer the entire sample mixture into a **Spin Filter Set GS** (filter with tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin filter GS** back into the **Receiver tube GS**

3	**DNA washing, step I**
	• Add 500 µl of **Wash Solution A** • 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 600 µl of **Wash Solution B** • 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 in order to remove remaining ethanol

5	**Elution of bacterial DNA**
	• Put the **Spin filter GS** into a new **1.5 ml Receiver tube** • Add 100 – 200 µl of **Elution buffer** (preheated at 65 °C) • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Store the eluted bacterial DNA at +4°C *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 further down-stream applications and can be directly used for PCR, real-time PCR, RFLP-analysis or stored at 4 °C for several weeks.

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

Warning !: Nexxo-Prep Bacteria DNA mini kit use a poly A **carrier RNA** and is not appropriated if DNA is directly used in gel electrophoresis or capillary electrophoresis.

Note: elution can also be carried out with ddH_2O.

VI. Protocol 3: bacterial DNA extraction from food samples

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter "Reagents and buffer solutions preparation", page 2).
- Check solutions for absence of precipitates before use. If necessary resuspend precipitates by heating (< 30 °C).
- Prepare adapted growth media for considered bacteria
- Preheat a heating block or water bath (37 °C, 65 °C and 95 °C will be required).
- 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).

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

	Preparation of food sample
1	• Take 25 g of food sample • Homogenise the sample • Add 225 ml of appropriate growth media (Fraser media for instance) • Carry out cell culture (e.g.: 24h) • Transfer 1 ml of the culture media in a tube • Centrifuge 3 min. at 11000 x g • Remove carefully the whole supernatant and only keep the pellet • Proceed with step 2a (Gram positive bacteria) or step 2b (Gram negative bacteria)

Depending on bacteria type, proceed with step 2a (Gram **+**) <u>or</u> step 2b (Gram **-**)

	Lysis of Gram positive bacteria
2a	• Add 400 µl of **Resuspension Buffer RSB** to the pellet • Resuspend the pellet by pipetting up and down • Transfer the entire suspension in an **Extraction tube** • Vortex briefly • Incubate in a heating block, 10 min. at 37 °C • Incubate in a heating block, 10 min. at 65 °C (if the transition from 37 °C to 65 °C is very time-consuming, increase incubation time by 2 min.) *Note: if the transition from 37 °C to 65 °C is longer than 7 min., increase incubation time by more than 2 min.* • Incubate **Extraction tube** in a heating block, 5 - 10 min. at 95 °C *Note: incubation under continuous shaking increase lysis efficiency* • Proceed with step 3 **« DNA adsorption to Spin filter GS »**

Steps 2b to 6 →

2b	**Lysis of Gram negative bacteria**
	• Add 400 µl of **Resuspension Buffer RSB** to the pellet • Resuspend the pellet by pipetting up and down • Transfer the entire suspension in an **Extraction tube** • Vortex briefly • Incubate in a heating block, 10 min. at 65 °C • Incubate **Extraction tube** in a heating block, 5 - 10 min. at 95 °C *Note: incubation under continuous shaking increase lysis efficiency* • Proceed with step 3 **« DNA adsorption to Spin filter GS »**

3	**DNA adsorption to Spin filter GS**
	• Add 400 µl of **Binding Solution XT** • Vortex briefly • Transfer the entire sample mixture into a **Spin Filter Set GS** (filter with tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin filter GS** back into the **Receiver tube GS**

4	**DNA washing, step I**
	• Add 500 µl of **Wash Solution A** • 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 600 µl of **Wash Solution B** • 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 in order to remove remaining ethanol

6	**Elution of bacterial DNA**
	• Put the **Spin filter GS** into a new **1.5 ml Receiver tube** • Add 100 – 200 µl of **Elution buffer** (preheated at 65 °C) • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Store the eluted bacterial DNA at +4°C *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 further down-stream applications and can be directly used for PCR, real-time PCR, RFLP-analysis or stored at 4 °C for several weeks.

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

Warning !: Nexxo-Prep Bacteria DNA mini kit use a poly A **carrier RNA** and is not appropriated if DNA is directly used in gel electrophoresis or capillary electrophoresis.

Note: elution can also be carried out with ddH_2O.

VII. Protocol 4: bacterial DNA extraction from paper points

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter “Reagents and buffer solutions preparation”, page 2).
- Check solutions for absence of precipitates before use. If necessary resuspend precipitates by heating (< 30 °C).
- Preheat a heating block or water bath (37 °C, 65 °C and 95 °C will be required).
- 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).

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

	Bacteria lysis
1	• Put the paper point into an **Extraction tube** • Add 400 µl of **Resuspension Buffer RSB** • Vortex briefly • Incubate in a heating block, 10 min. at 37 °C • Incubate in a heating block, 10 min. at 65 °C (if the transition from 37 °C to 65 °C is very time-consuming, increase incubation time by 2 min.) *Note: if the transition from 37 °C to 65 °C is longer than 7 min., increase incubation time by more than 2 min.* • Incubate **Extraction tube** in a heating block, 5 - 10 min. at 95 °C *Note: incubation under continuous shaking increase lysis efficiency* • Centrifuge 1 min. at max. speed • Transfer the whole supernatant into a 1.5 ml tube (not supplied). *Note: don’t transfer the pellet (the paper point)*

	DNA adsorption to Spin filter GS
2	• Add 400 µl of **Binding Solution XT** to the tube containing the supernatant • Vortex briefly • Transfer the sample mixture into a **Spin Filter Set GS** (filter with tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin filter GS** back into the **Receiver tube GS**

	DNA washing, step I
3	• Add 500 µl of **Wash Solution A** • 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**

Steps 4 to 5 →

4	**DNA washing, step II** • Add 600 µl of **Wash Solution B** • 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 in order to remove remaining ethanol

5	**Elution of bacterial DNA** • Put the **Spin filter GS** into a new **1.5 ml Receiver tube** • Add 200 µl of **Elution buffer** (preheated at 65 °C) *Note: when 3 or 4 paper points were used, increase **Elution buffer** volume by 200 µl* • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Store the eluted bacterial DNA at +4°C *Note: depending on desired yield and concentration, DNA can be eluted with more or less (min. 50 µl) elution buffer.* *Higher yield is reached when eluting twice.*

Eluted DNA is ready for further down-stream applications and can be directly used for PCR, real-time PCR, RFLP-analysis or stored at 4 °C for several weeks.

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

Warning !: Nexxo-Prep Bacteria DNA mini kit use a poly A **carrier RNA** and is not appropriated if DNA is directly used in gel electrophoresis or capillary electrophoresis.

Note: elution can also be carried out with ddH_2O.

VIII. Protocol 5: bacterial DNA extraction from tissue biopsies (1 - 10 mg)

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter "Reagents and buffer solutions preparation", page 2).
- Check solutions for absence of precipitates before use. If necessary resuspend precipitates by heating (< 30 °C).
- Preheat a heating block or water bath (56 °C and 95 °C will be required).
- 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).

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

1	**Tissue lysis** • Transfer 1 – 10 mg tissue into an **Extraction tube** • Add 400 µl of **Resuspension Buffer RSB** • Vortex briefly (cap closed) • Incubate 30 – 60 min. at 56 °C, under continuous shacking *Note: increase incubation time until lysis is complete* • Incubate **Extraction tube** in a heating block, 5 - 10 min. at 95 °C *Note: incubation under continuous shaking increase lysis efficiency* • Centrifuge 1 min. at max. speed • Transfer the whole supernatant into a 1.5 ml tube (not supplied) *Note: don't transfer the pellet*

2	**DNA adsorption to Spin filter GS** • Add 400 µl of **Binding Solution XT** to the tube containing the supernatant • Vortex briefly • Transfer the sample mixture into a **Spin Filter Set GS** (filter with tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin filter GS** back into the **Receiver tube GS**

3	**DNA washing, step I** • Add 500 µl of **Wash Solution A** • 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**

Steps 4 to 5 →

4	**DNA washing, step II** • Add 600 µl of **Wash Solution B** • 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 in order to remove remaining ethanol

5	**Elution of bacterial DNA** • Put the **Spin filter GS** into a new **1.5 ml Receiver tube** • Add 80 – 120 µl of **Elution buffer** (preheated at 65 °C) • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Store the eluted bacterial DNA at +4°C *Note: depending on desired yield and concentration, DNA can be eluted with more or less (min. 50 µl) elution buffer.*

Eluted DNA is ready for further down-stream applications and can be directly used for PCR, real-time PCR, RFLP-analysis or stored at 4 °C for several weeks.

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

Warning !: Nexxo-Prep Bacteria DNA mini kit use a poly A **carrier RNA** and is not appropriated if DNA is directly used in gel electrophoresis or capillary electrophoresis.

Note: elution can also be carried out with ddH_2O.

IX. Protocol 6: bacterial DNA extraction from paraffin embedded tissue

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter "Reagents and buffer solutions preparation", page 2).
- Check solutions for absence of precipitates before use. If necessary resuspend precipitates by heating (< 30 °C).
- Preheat a heating block or water bath (56 °C and 95 °C will be required).
- 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).
- The first step needs octane and >96 % ethanol.

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

	Dewaxing
1	• 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 max. speed • Remove delicately the supernatant *Note: repeat this step until paraffin is completely removed* • Add 0.5 ml of >96 % ethanol to the pellet • Mix completely • Centrifuge briefly • Remove ethanol with a pipette • Incubate the open tube at 56 °C to remove the remaining ethanol

	Tissue lysis
2	• Transfer the dewaxed tissue into an **Extraction tube** • Add 400 µl of **Resuspension Buffer RSB** • Vortex briefly (cap closed) • Incubate 30 – 60 min. at 56 °C, under continuous shacking *Note: increase incubation time until lysis is complete* • Incubate **Extraction tube** in a heating block, 5 - 10 min. at 95 °C *Note: incubation under continuous shaking increase lysis efficiency* • Centrifuge 1 min. at max. speed • Transfer the whole supernatant into a 1.5 ml tube (not supplied) *Note: don't transfer the pellet*

Steps 3 to 6 →

3	**DNA adsorption to Spin filter GS**
	• Add 400 µl of **Binding Solution XT** to the tube containing the supernatant • Vortex briefly • Transfer the sample mixture into a **Spin Filter Set GS** (filter with tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin filter GS** back into the **Receiver tube GS**

4	**DNA washing, step I**
	• Add 500 µl of **Wash Solution A** • 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 600 µl of **Wash Solution B** • 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 in order to remove remaining ethanol

6	**Elution of bacterial DNA**
	• Put the **Spin filter GS** into a new **1.5 ml Receiver tube** • Add 80 – 120 µl of **Elution buffer** (preheated at 65 °C) • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Store the eluted bacterial DNA at +4°C *Note: depending on desired yield and concentration, DNA can be eluted with more or less (min. 50 µl) elution buffer.*

Eluted DNA is ready for further down-stream applications and can be directly used for PCR, real-time PCR, RFLP-analysis or stored at 4 °C for several weeks.

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

Warning !: Nexxo-Prep Bacteria DNA mini kit use a poly A **carrier RNA** and is not appropriated if DNA is directly used in gel electrophoresis or capillary electrophoresis.

Note: elution can also be carried out with ddH_2O.

X. Protocol 7: bacterial DNA extraction from urine sample

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter "Reagents and buffer solutions preparation", page 2).
- Check solutions for absence of precipitates before use. If necessary resuspend precipitates by heating (< 30 °C).
- Preheat a heating block or water bath (37 °C, 65 °C and 95 °C will be required).
- 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).

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

	Preparation of the urine sample
1	• Transfer 15 – 50 ml of the urine sample in an appropriate tube • Centrifuge 15 min. at 1300 x g • Carefully decant the supernatant, without disturbing the pellet (sediment) • Resuspend the pellet (sediment) with 3 ml of 1X PBS • Centrifuge 5 min. at 1300 x g • Carefully decant the whole supernatant by inverting the tube, then keep the tube inverted a few minutes • Proceed with step 2a (Gram positive bacteria) or step 2b (Gram negative bacteria)

Depending on bacteria type, proceed with step 2a (Gram **+**) or step 2b (Gram **-**)

	Lysis of Gram positive bacteria
2a	• Add 400 µl of **Resuspension Buffer RSB** to the pellet • Resuspend the pellet by pipetting up and down • Transfer the entire suspension in an **Extraction tube** • Vortex briefly • Incubate in a heating block, 10 min. at 37 °C • Incubate in a heating block, 10 min. at 65 °C (if the transition from 37 °C to 65 °C is very time-consuming, increase incubation time by 2 min.) *Note: if the transition from 37 °C to 65 °C is longer than 7 min., increase incubation time by more than 2 min.* • Incubate **Extraction tube** in a heating block, 5 - 10 min. at 95 °C *Note: incubation under continuous shaking increase lysis efficiency* • Proceed with step 3 **« DNA adsorption to Spin filter GS »**

Steps 2b to 6 →

2b	**Lysis of Gram negative bacteria**
	• Add 400 µl of **Resuspension Buffer RSB** to the pellet • Resuspend the pellet by pipetting up and down • Transfer the entire suspension in an **Extraction tube** • Vortex briefly • Incubate in a heating block, 10 min. at 65 °C • Incubate **Extraction tube** in a heating block, 5 - 10 min. at 95 °C *Note: incubation under continuous shaking increase lysis efficiency* • Proceed with step 3 **« DNA adsorption to Spin filter GS »**

3	**DNA adsorption to Spin filter GS**
	• Add 400 µl of **Binding Solution XT** • Vortex briefly • Transfer the entire sample mixture into a **Spin Filter Set GS** (filter with tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin filter GS** back into the **Receiver tube GS**

4	**DNA washing, step I**
	• Add 500 µl of **Wash Solution A** • 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 600 µl of **Wash Solution B** • 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 in order to remove remaining ethanol

6	**Elution of bacterial DNA**
	• Put the **Spin filter GS** into a new **1.5 ml Receiver tube** • Add 100 µl of **Elution buffer** (preheated at 65 °C) • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Store the eluted bacterial DNA at +4°C *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 further down-stream applications and can be directly used for PCR, real-time PCR, RFLP-analysis or stored at 4 °C for several weeks.

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

Warning !: Nexxo-Prep Bacteria DNA mini kit use a poly A **carrier RNA** and is not appropriated if DNA is directly used in gel electrophoresis or capillary electrophoresis.

Note: elution can also be carried out with ddH_2O.

XI. Protocol 8: bacterial DNA extraction from water sample (more than 1 liter)

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter "Reagents and buffer solutions preparation", page 2).
- Check solutions for absence of precipitates before use. If necessary resuspend precipitates by heating (< 30 °C).
- Preheat a heating block or water bath (37 °C, 65 °C and 95 °C will be required).
- 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).

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

	Preparation of the water sample
1	• Use a standard approach (filtration, centrifugation …) to concentrate the water sample (e.g. 1 liter) • Centrifuge in a 50 ml tube to get a pellet • Carefully decant the supernatant, without disturbing the pellet (sediment) • Resuspend the pellet (sediment) with 10 ml of 1X PBS • Centrifuge 5 min. at 1300 x g • Carefully decant the whole supernatant by inverting the tube, then keep the tube inverted a few minutes • Proceed with step 2a (Gram positive bacteria) or step 2b (Gram negative bacteria)

Depending on bacteria type, proceed with step 2a (Gram **+**) or step 2b (Gram **-**)

	Lysis of Gram positive bacteria
2a	• Add 400 µl of **Resuspension Buffer RSB** to the pellet • Resuspend the pellet by pipetting up and down • Transfer the entire suspension in an **Extraction tube** • Vortex briefly • Incubate in a heating block, 10 min. at 37 °C • Incubate in a heating block, 10 min. at 65 °C (if the transition from 37 °C to 65 °C is very time-consuming, increase incubation time by 2 min.) *Note: if the transition from 37 °C to 65 °C is longer than 7 min., increase incubation time by more than 2 min.* • Incubate **Extraction tube** in a heating block, 5 - 10 min. at 95 °C *Note: incubation under continuous shaking increase lysis efficiency* • Proceed with step 3 **« DNA adsorption to Spin filter GS »**

Steps 2b to 6 →

2b	**Lysis of Gram negative bacteria**
	• Add 400 µl of **Resuspension Buffer RSB** to the pellet • Resuspend the pellet by pipetting up and down • Transfer the entire suspension in an **Extraction tube** • Vortex briefly • Incubate in a heating block, 10 min. at 65 °C • Incubate **Extraction tube** in a heating block, 5 - 10 min. at 95 °C *Note: incubation under continuous shaking increase lysis efficiency* • Proceed with step 3 **« DNA adsorption to Spin filter GS »**

3	**DNA adsorption to Spin filter GS**
	• Add 400 µl of **Binding Solution XT** • Vortex briefly • Transfer the entire sample mixture into a **Spin Filter Set GS** (filter with tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **Spin filter GS** back into the **Receiver tube GS**

4	**DNA washing, step I**
	• Add 500 µl of **Wash Solution A** • 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 600 µl of **Wash Solution B** • 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 in order to remove remaining ethanol

6	**Elution of bacterial DNA**
	• Put the **Spin filter GS** into a new **1.5 ml Receiver tube** • Add 100 µl of **Elution buffer** (preheated at 65 °C) • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Store the eluted bacterial DNA at +4°C *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 further down-stream applications and can be directly used for PCR, real-time PCR, RFLP-analysis or stored at 4 °C for several weeks.

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

Warning !: Nexxo-Prep Bacteria DNA mini kit use a poly A **carrier RNA** and is not appropriated if DNA is directly used in gel electrophoresis or capillary electrophoresis.

Note: elution can also be carried out with ddH_2O.