# Nexxo-Prep Plasmide mini

DNA extraction, by spin-column system, for the isolation, in less than 15 minutes, of up to 20 µg plasmid DNA from 0.5 – 2.0 ml bacterial culture.

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

## I. Kit components

| | 10 preps | 250 preps |
|---|---|---|
| **Tampon d'élution P** (Elution Buffer P) | 2 ml | 30 ml |
| **Solution de lavage SLB** (Wash Solution SLB) | 10 ml (ready-to-use) | 40 ml (final volume: 200 ml) |
| **Tampon de resuspension** (Resuspension Buffer) | 2 x 2 ml | 70 ml |
| **Tampon de lyse L** (Lysis Buffer L) | 2 x 2 ml | 70 ml |
| **Tampon de neutralisation N** (Neutralization Buffer N) | 2 x 2 ml | 70 ml |
| **Filtres de centrifugation** (Spin Filter) | 10 | 5 x 50 |
| **Tubes receveurs 1,5 ml** (1,5 ml Receiver Tubes) | 10 | 5 x 50 |
| **Tubes receveurs 2,0 ml** (2,0 ml Receiver Tubes) | 10 | 5 x 50 |
| **User guide** | 1 | 1 |
| **Art. No.** | 2036.10 | 2036.250 |

### Required material and equipment not included in this kit

- Ethanol >96 %
- Reaction tubes (1.5 ml / 2.0 ml)
- Microcentrifuge (12000 - 16000 x g)
- Pipettes with corresponding tips
- Disposable gloves
- Lysozyme (only for protocol 3)

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 is a volatile compound. Keep **Wash Solution SLB** tightly closed.

Check solutions for absence of precipitates before use. Redisolve precipitates by heating (< 37 °C).

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

## III. Reagents and buffer solutions preparation

1. Kit 10 extractions:

   All components are delivered ready-to-use.

2. Kit 250 extractions:

   Add 160 ml of >96 % ethanol to the **Wash Solution SLB** and store the bottle tightly closed.

# IV. Protocol 1: plasmid extraction from 0.5 - 2.0 ml bacterial culture (ex.: *E. coli*)

## 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 (< 37 °C).

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

| | |
|---|---|
| 1 | **Preparation of bacterial culture**<br>• Inoculate 1 – 5 ml of LB broth (supplemented with the appropriate selective antibiotic), with a single colony<br>• Incubate 12-16 hours at 37 °C with rapid shaking<br>*Note: incubation period should not be longer than 16 hours.* |

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| 2 | **Bacterial cells harvesting**<br>• Transfer 0.5 - 2.0 ml of bacterial culture in a 1.5 or 2.0 ml reaction tube<br>• Centrifuge 1 min. at max. speed (12000 - 16000 x g)<br>• Discard the whole supernatant, and keep only the pellet |

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| 3 | **Resuspension of bacterial cells**<br>• Add 250 µl of **Resuspension Buffer**<br>• Vortex until pellet is completely resuspended (any pellets or cell clumps should remain) |

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| 4 | **Cell lysis and neutralization**<br>• Add 250 µl of **Lysis Buffer L**<br>• Close the tube and mix gently by inversion (5 inversions, do not vortex)<br>Caution: lysis step (↑) should not take more than 5 minutes.<br>• Add 250 µl of **Neutralization Buffer N**<br>• Mix by inversion (mix gently but effectively, 4 – 6 inversions)<br>• Centrifuge 5 min. at max. speed (12000 - 16000 x g) |

Steps 5 to 7 →

| 5 | **Plasmid DNA adsorption to Spin Filter** |
|---|---|
| | • Insert a **Spin Filter** into a new **2,0 ml Receiver Tube**<br>• Transfer the clarified supernatant into the **Spin Filter**<br>• Incubate 1 min. at room temperature<br>• Centrifuge 1 min. at 11000 x g<br>• Discard the flow-through and put the **Spin filter** back into the **2.0 ml Receiver tube** |

| 6 | **Plasmid DNA washing** |
|---|---|
| | • Add 750 µl of **Wash Solution SLB** to the **Spin Filter**<br>• Centrifuge 1 min. at 11000 x g<br>• Discard the flow-through and put the **Spin filter** back into the **2.0 ml Receiver tube**<br>• Centrifuge 3 min. at max. speed (12000 - 16000 x g), to remove remaining ethanol from the **Spin Filter** |

| 7 | **Plasmid DNA elution** |
|---|---|
| | • Insert the **Spin filter** into a new **1.5 ml Receiver tube**<br>• Add 50-100 µl of **Elution Buffer P** onto the center of the **Spin Filter**<br>• Incubate 1 min. at room temperature<br>• Centrifuge 1 min. at 11000 x g, to elute plasmid DNA<br><br>*Note: depending on desired yield and concentration, DNA can be eluted with more or less elution buffer.*<br><br>*Higher yield is reached when eluting twice (2 x 100 µl for instance).* |

Eluted plasmid DNA is ready for down-stream applications and can directly be used for PCR, sequencing, enzymatic digestion, cloning, etc., or can be stored at 2 °C to 8 °C.

Storing pDNA at -15 to -25°C might be damaging (shearing of pDNA). Repeated freeze-thaw cycles enhance the risk of pDNA degradation.

*Note: **Elution buffer P** contains EDTA. Elution can also be achieved with dd$H_2O$. Without buffer pDNA may deteriorate. If pDNA is eluted with dd$H_2O$ it is recommended to store pDNA at -20 °C.*

# V. Protocol 2: extraction of low copy plasmids and cosmids, from up to 10 ml bacterial culture

*Note: this protocol needs a higher volume of buffers. Using this protocol reduces the 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 (< 37 °C).
- For plasmids or cosmids with more than 10 kb, the last step needs **Elution Buffer P** (or $ddH_2O$, as the case may be) heated at 70 °C. Warm up in due time required volume of **Elution Buffer P** in a 2 ml tube (tube not supplied).

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

| | |
|---|---|
| 1 | **Preparation of bacterial culture**<br>• Inoculate 1 – 10 ml of LB broth (supplemented with the appropriate selective antibiotic), with a single colony<br>• Incubate 12-16 hours at 37 °C with rapid shaking<br>*Note: incubation period should not be longer than 16 hours.* |
| 2 | **Bacterial cells harvesting**<br>• Transfer 1 - 10 ml of bacterial culture in a 15 ml Falcon reaction tube<br>• Centrifuge 10 min. at 5400 x g and 4 °C<br>• Discard the whole supernatant, and keep only the pellet |
| 3 | **Resuspension of bacterial cells**<br>• Add 500 µl of **Resuspension Buffer**<br>• Vortex until pellet is completely resuspended (any pellets or cell clusters should remain)<br>• Transfer the whole solution in a 2.0 ml reaction tube (not supplied) |

Steps 4 to 7 →

| 4 | **Cell lysis and neutralization** |
|---|---|
| | • Add 500 µl of **Lysis Buffer L**<br>• Close the tube and mix gently by inversion (5 inversions, do not vortex)<br>Caution: lysis step (↑) should not take more than 5 minutes.<br>• Add 500 µl of **Neutralization Buffer N**<br>• Mix by inversion (mix gently but effectively, 4 – 6 inversions)<br>• Centrifuge 5 min. at max. speed (12000 - 16000 x g) |

| 5 | **Plasmid DNA adsorption to Spin Filter** |
|---|---|
| | • Insert a **Spin Filter** into a new **2,0 ml Receiver Tube**<br>• Transfer 750 µl of clarified supernatant, from the previous step, into the **Spin Filter**<br>• Incubate 1 min. at room temperature<br>• Centrifuge 1 min. at 11000 x g<br>• Discard the flow-through and put the **Spin filter** back into the **2.0 ml Receiver tube**<br>• Transfer the leftover clarified supernatant, from step 4, into the **Spin Filter**<br>• Incubate 1 min. at room temperature<br>• Centrifuge 1 min. at 11000 x g<br>• Discard the flow-through and put the **Spin filter** back into the **2.0 ml Receiver tube** |

| 6 | **Plasmid DNA washing** |
|---|---|
| | • Add 750 µl of **Wash Solution SLB** to the **Spin Filter**<br>• Centrifuge 1 min. at 11000 x g<br>• Discard the flow-through and put the **Spin filter** back into the **2.0 ml Receiver tube**<br>• Centrifuge 3 min. at max. speed (12000 - 16000 x g), to remove remaining ethanol from the **Spin Filter** |

| 7 | **Plasmid DNA elution** |
|---|---|
| | • Insert the **Spin filter** into a new **1.5 ml Receiver tube**<br>• Add 50-100 µl of **Elution Buffer P** (*) onto the center of the **Spin Filter**<br>(*) For plasmids or cosmids with more than 10 kb, use **Elution Buffer P** heated at 70 °C<br>• Incubate 1 min. at room temperature<br>• Centrifuge 1 min. at 11000 x g, to elute plasmid DNA<br>*Note: depending on desired yield and concentration, DNA can be eluted with more or less elution buffer.*<br>*Higher yield is reached when eluting twice (2 x 100 µl for instance).* |

Eluted plasmid DNA is ready for down-stream applications and can directly be used for PCR, sequencing, enzymatic digestion, cloning, etc., or can be stored at 2 °C to 8 °C.

Storing pDNA at -15 to -25°C might be damaging (shearing of pDNA). Repeated freeze-thaw cycles enhance the risk of pDNA degradation.

*Note: **Elution buffer P** contains EDTA. Elution can also be achieved with $ddH_2O$. Without buffer pDNA may deteriorate. If pDNA is eluted with $ddH_2O$ it is recommended to store pDNA at -20 °C.*

# VI. Protocol 3: plasmid extraction from 0.5 - 2.0 ml Gram positive bacterial culture

## 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 (< 37 °C).
- Prepare 10 µl of Lysozyme (10 mg/ml or according to manufacturer’s instructions) for step 3 (Resuspension of bacterial cells)

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

| | |
|---|---|
| 1 | **Preparation of bacterial culture**<br>• Inoculate 1 – 5 ml of LB broth (supplemented with the appropriate selective antibiotic), with a single colony<br>• Incubate 12-16 hours at 37 °C with rapid shaking<br>*Note: incubation period should not be longer than 16 hours.* |

| | |
|---|---|
| 2 | **Bacterial cells harvesting**<br>• Transfer 0.5 - 2.0 ml of bacterial culture in a 1.5 or 2.0 ml reaction tube<br>• Centrifuge 1 min. at max. speed (12000 - 16000 x g)<br>• Discard the whole supernatant, and keep only the pellet |

| | |
|---|---|
| 3 | **Resuspension of bacterial cells**<br>• Add 250 µl of **Resuspension Buffer**<br>• Vortex until pellet is completely resuspended (any pellets or cell clusters should remain)<br>• Add 10 µl of Lysozyme (10 mg/ml or according to manufacturer’s instructions)<br>• Vortex<br>• Incubate 10 min. at 37 °C |

Steps 4 to 7 →

| 4 | **Cell lysis and neutralization** |
|---|---|
| | • Add 250 µl of **Lysis Buffer L**<br>• Close the tube and mix gently by inversion (5 inversions, do not vortex)<br>Caution: lysis step (↑) should not take more than 5 minutes.<br>• Add 250 µl of **Neutralization Buffer N**<br>• Mix by inversion (mix gently but effectively, 4 – 6 inversions)<br>• Centrifuge 5 min. at max. speed (12000 - 16000 x g) |

| 5 | **Plasmid DNA adsorption to Spin Filter** |
|---|---|
| | • Insert a **Spin Filter** into a new **2,0 ml Receiver Tube**<br>• Transfer the clarified supernatant into the **Spin Filter**<br>• Incubate 1 min. at room temperature<br>• Centrifuge 1 min. at 11000 x g<br>• Discard the flow-through and put the **Spin filter** back into the **2.0 ml Receiver tube** |

| 6 | **Plasmid DNA washing** |
|---|---|
| | • Add 750 µl of **Wash Solution SLB** to the **Spin Filter**<br>• Centrifuge 1 min. at 11000 x g<br>• Discard the flow-through and put the **Spin filter** back into the **2.0 ml Receiver tube**<br>• Centrifuge 3 min. at max. speed (12000 - 16000 x g), to remove remaining ethanol from the **Spin Filter** |

| 7 | **Plasmid DNA elution** |
|---|---|
| | • Insert the **Spin filter** into a new **1.5 ml Receiver tube**<br>• Add 50-100 µl of **Elution Buffer P** onto the center of the **Spin Filter**<br>• Incubate 1 min. at room temperature<br>• Centrifuge 1 min. at 11000 x g, to elute plasmid DNA<br>*Note: depending on desired yield and concentration, DNA can be eluted with more or less elution buffer.*<br>*Higher yield is reached when eluting twice (2 x 100 µl for instance).* |

Eluted plasmid DNA is ready for down-stream applications and can directly be used for PCR, sequencing, enzymatic digestion, cloning, etc., or can be stored at 2 °C to 8 °C.

Storing pDNA at -15 to -25°C might be damaging (shearing of pDNA). Repeated freeze-thaw cycles enhance the risk of pDNA degradation.

*Note: **Elution buffer P** contains EDTA. Elution can also be achieved with dd$H_2O$. Without buffer pDNA may deteriorate. If pDNA is eluted with dd$H_2O$ it is recommended to store pDNA at -20 °C.*