XpressBio
Life Science Products

nexttec™
DNA isolation systems

Protocol

Plasmid DNA

from Bacteria (E.coli)

by nexttec™ 1-Step

- nexttec™ cleanPlate96 -

Cat. No. 30N.901

Cat. No. 30N.902

Cat. No. 30N.904

Cat. No. 30N.924

Version 1.0

For research only

Principle

nexttec™1^{-Step} is the easiest handling and fasted DNA purification system containing a single buffer system and a one-step DNA purification after lysis.
Proteins, detergents and low molecular weight compounds are retained by the nexttec™ sorbent. DNA passes through the nexttec™ cleanPlate96 during a short, one-step purification procedure.
The obtained DNA is suitable for all common enzymatic reactions (restriction digests, real-time PCR, PCR, genotyping etc.).

Kit contents

The kit contains all necessary reagents for lysis and subsequent DNA purification.

Component	**Art.No. 30N.901**	**Art.No. 30N.902**	**Art.No. 30N.904**	**Art.No. 30N.924**
Buffer P	55 ml	55 ml	75 ml	390 ml
Protease	2 ml	2 ml	3 ml	16 ml
Prep Solution	100 ml	100 ml	150 ml	2x 450 ml
RNase A	5 ml	5 ml	7 ml	42 ml
Lysozyme	37.5 mg (1.5 ml)	37.5 mg (1.5 ml)	50 mg (2.0 ml)	250 mg (10 ml)
nexttec™ cleanPlates96	1	2	4	24
nexttec™ deep-well plates	3	6	12	72
Sealing tapes	4	8	16	96
Alu sealing tapes	2	4	8	48

nexttec™ service

For extending the application range to tissues, which are difficult to lyse by the standard procedure, it is recommended to include optional components in the lysis buffer, optimizing the lysis time or using different lysis volumes (e.g. robotic applications).
For detailed information, please get in contact with **service@nexttec.biz**.

Storage Conditions

During shipment all kit components are stable at room temperature. After arrival, **Buffer P, Protease, Prep Solution, RNase A** and **Lysozyme must** be stored at **+2°C to +8°C**.
nexttec™cleanPlates96 can be stored at **room temperature (+20°C to +25°C)**. If properly stored, see expiration date for the stability of the kit.

Safety Information

Protease	Xn	R 36/37/38 R42/43; S 23-24-26 36/37

Risk Phrases

R 36/37/38: Irritating to eyes, respiratory system and skin.
R 42/43: May cause sensitisation by inhalation and skin contact.

Safety Phrases

S 23: Do not breathe gas/fumes/vapour/spray.
S 24: Avoid contact with skin.
S 26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S 36/37: Wear suitable protective clothing and gloves.

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information please consult the appropriate material safety data sheets (MSDS).

Before starting

- **Equilibrate nexttec™ cleanPlates96**

E1	Add **350 µl Prep Solution** onto each well of a **nexttec™ cleanPlate96**. Incubate for at least **5 min** at room temperature and centrifuge at **350x g** for **1 min** or apply vacuum for **30 to 60 sec** to remove excess buffer.
E2	Discard the deep-well plate. Place the **nexttec™ cleanPlate96** onto a new deep-well plate. Use equilibrated **nexttec™ cleanPlates96** or store closed **at +2°C to +8°C** and use within one week.

- **Preheat a water bath to 90°C**

- **Dissolve Lysozyme**

  Add **1.5 ml** (30N.901 / 30N.902), **2.0 ml** (30N.904) or **10 ml** (30N.924) of **purified water** to the **lyophilized lysozyme** (final concentration **25 mg/ml**), mix thoroughly by vortexing and **store at -18°C to -25°C.**

Protocol

Lysis

L1	Transfer **1 ml** bacteria overnight cultures to a deep-well plate. Centrifuge (**2.000x g, 10 min**), carefully remove and discard supernatant.
L2	Add **130 µl Buffer P**, **5 µl Protease** and **3 µl Lysozyme*** to the cell pellet. Dissolve cell pellets by pipetting up and down, close with an alu sealing tape. Incubate for **5 min** at **room temperature**.
L3	Transfer plate to a preheated water bath (**90°C**). Incubate for **1 min**. Cool down plate to **room temperature.**
L4	Add **15 µl RNase A** to each lysate. Mix gently (do not vortex!), Incubate for **3 min** at **room temperature**.

*For Pre-Mixes see Technical Section.

Purification of DNA

P	Transfer the complete **lysate** onto an **equilibrated nexttec™ cleanPlate96** (use 1 ml or 200 µl wide bore pipette tips). Incubate for **3 min** at **room temperature**. Centrifuge at **700x g** for **1 min** or apply vacuum for **1 min**.
	The eluate contains the purified DNA!!

Notes:

Technical Section

- **Preparation of Lysis buffers (Pre-Mixes)**

LP	**Lysis Buffer LP**:	1 sample	1 plate	2 plates	3 plates	4 plates
	Buffer P	130 µl	31.8 ml	63.6 ml	95.4 ml	127.2 ml
	Protease	5 µl	0.6 ml	1.2 ml	1.8 ml	2.4 ml
	Lysozyme	3 µl	0.36 ml	0.72 ml	1.08 ml	1.44 ml
	Mix by vortexing. Add **138 µl** of **Lysis Buffer LP** to each sample (L2). The **Lysis Buffer LP** is stable for **1** working day, if stored **at +2°C to +8°C**.					

- **Determination of DNA concentration in nexttec™ 1-Step DNA preparations**

We recommend determining the DNA concentration:

- Using the fluorescent dye Picogreen® or similar

- Comparing the fluorescence intensity of DNA bands of unknown concentration with standards, e.g. in ethidium bromide stained agarose gels.

Please notice:
The use of absorption measurement at 260 nm (A_{260}) in a spectrophotometer (e.g. NanoDrop®) for determination of DNA concentration is system related not recommended. For details and possible workarounds for your specific application please contact:
service@nexttec.biz.

- **Centrifugation**

For centrifugation at 350x g or 700x g use the settings for relative centrifugal force (RCF) of your centrifuge. Alternatively measure the distance of the nexttec™ cleanPlate96 to the centre of your rotor and calculate the necessary rotations per minute (e.g. rpm = 299.07 x $\sqrt{350/r}$; r=radius in cm)

- **Vacuum Application**

Assemble the vacuum manifold as described in the manual. The flow rate should be between 15 L/min and 150 L/min.
A regulation of vacuum is not necessary.

Product Use Restriction

nexttec™ 1-Step DNA Isolation Kit components were developed, designed and sold **for research purposes only**. They are suitable for in vitro uses only. No claim or representation is intended for use to identify any specific organism or of clinical use.
It is the responsibility of the user to verify the use of the nexttec™ 1-Step DNA Isolation Kit for a specific application as the performance characteristic of this kit has not been verified to a specific organism.

Troubleshooting, FAQ and Special Applications

Product claims are subject to change. Therefore, please, visit our website or contact our technical service team for troubleshooting guide, up-to-date protocols and latest applications on nexttec™ 1-Step products.

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