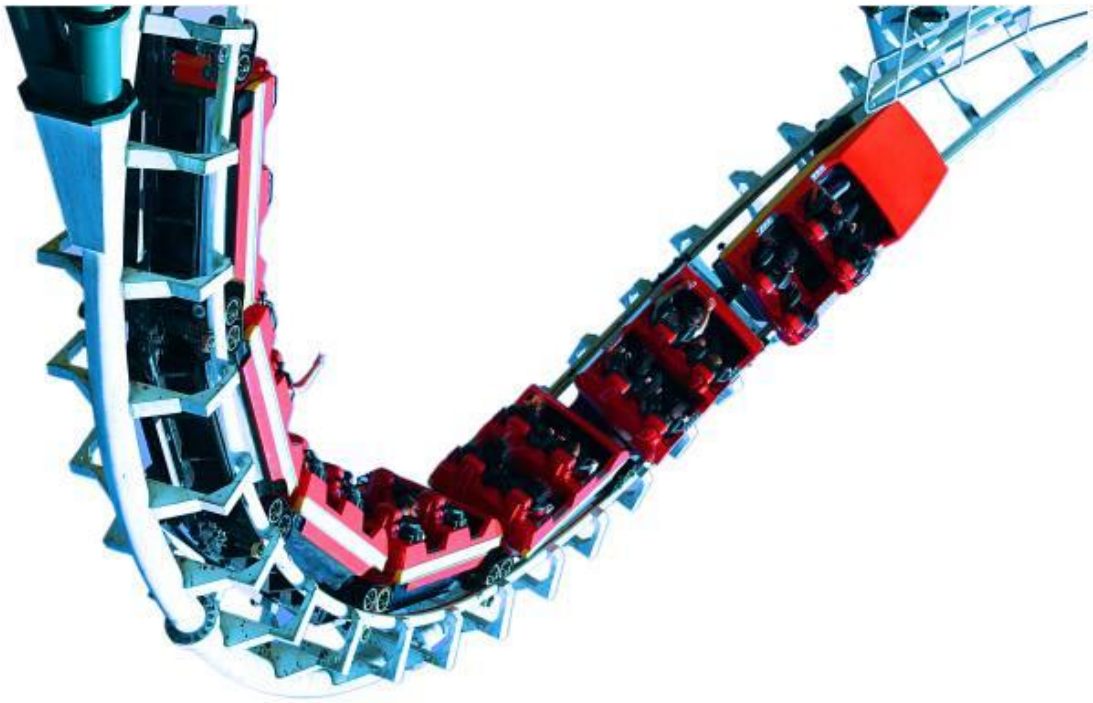

nexttec™

DNA isolation systems

Protocol

Plasmid DNA
from Bacteria (*E. coli*)
by nexttec™ 1-Step

- nexttec™ cleanPlate96 -

Cat. No. 30N.901	393091N
Cat. No. 30N.902	393092N
Cat. No. 30N.904	393094N
Cat. No. 30N.924	393096N

Version 3.0

For research only

Biozym
SCIENCE IS OUR BUSINESS

Principle

nexttec™ 1-Step is the easiest handling and fastest 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™ cleanColumn 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	2 x 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 plate	3	6	12	72
Sealing tapes	4	8	16	96
Alu sealing tapes	2	4	8	48

nexttec™ service

To extend the application range to samples, which are difficult to lyse by the standard procedure, it is recommended to include optional components in the lysis buffer and to optimize the lysis conditions. Please get in contact with **service@nexttec.biz** for detailed information.

Storage Conditions

During shipment all kit components are stable at room temperature. After arrival, store **Buffer P, Protease, RNase A** and **Prep Solution** at **+2 °C to +8 °C. Lysozyme** must be stored at **-18 °C to -25 °C.**
nexttec™ cleanPlates96 are stored at **room temperature (+20 °C to +25 °C)**. If properly stored, see expiration date for the stability of the kit.

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Safety Information

Protease Danger H315, H318, H334, H335

P261, P280, P342+P311, P305+P351+P338

Hazard Statements

H315 Causes skin irritation
H318 Causes serious eye irritation
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled
H335 May cause respiratory irritation

Precautionary Statements

P280	Wear protective gloves/protective clothing/eye protection/ face protection
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
P305+P351+ P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P261	Avoid breathing dust/fume/gas/mist/vapors/spray

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 **350 x g** for **1 min** or apply vacuum for **30 to 60 sec** to remove excess buffer.
E2	Discard the Waste 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 an incubator 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.**

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Protocol

Lysis

L1	Transfer **1 ml** bacteria overnight cultures to a deep-well plate. Centrifuge (**2,000x g, 10 min**), carefully remove and discard the 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 plate 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:

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Technical Section

Preparation of Lysis Pre-Mix

LP	**Lysis Buffer LP**:	1 sample	1 plate	2 plates	4 plates
	Buffer P	130 µl	14.3 ml	28.6 ml	57.5 ml
	Protease	5 µl	550 µl	1.1 ml	2.2 ml
	Lysozyme	3 µl	330 µl	660 µl	1.32 ml
	Mix by vortexing. Add **138 µl** of **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 to determine the DNA concentration:

- Using the fluorescent dye Picogreen® or similar.

- Comparing the fluorescence intensity of DNA bands of unknown concentrations 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 for clinical use.

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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.

Contact Information

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Ordering Information

For ordering information please visit our website **www.nexttec.biz**.

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