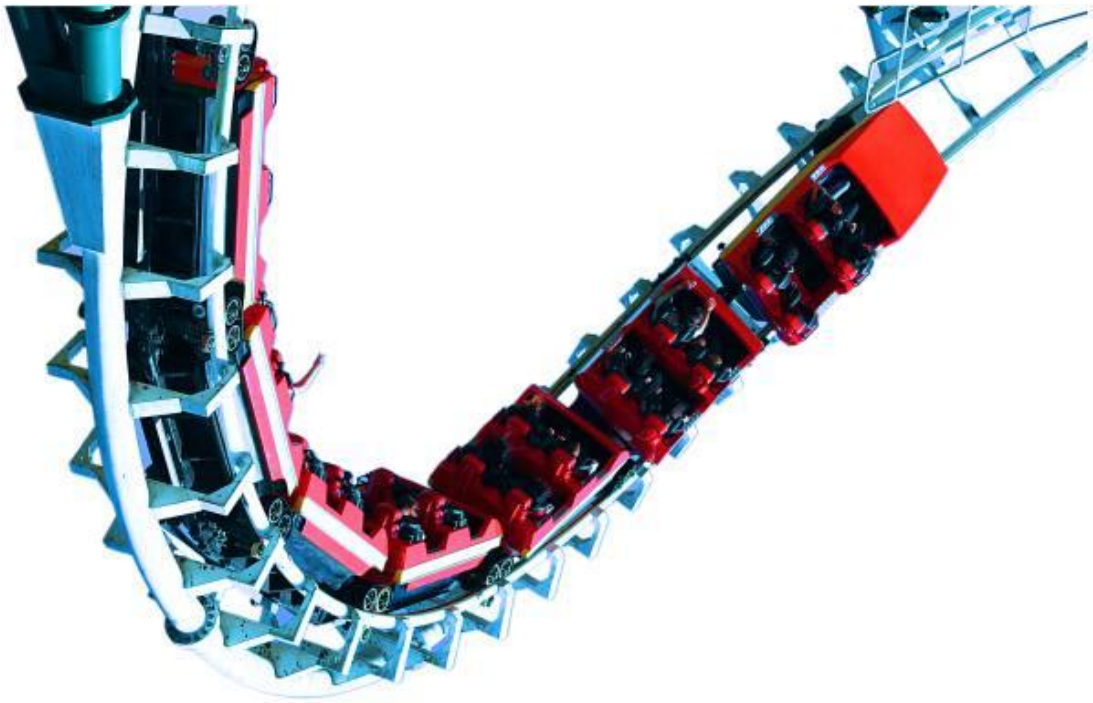

nexttec™

DNA isolation systems

Protocol

DNA Isolation from Bacteria by nexttec™ 1-Step

- nexttec™ cleanPlate96 -

Cat. No. 20N.901	392091N
Cat. No. 20N.902	392092N
Cat. No. 20N.904	391094N
Cat. No. 20N.924	391096N

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. 20N.901	Art.No. 20N.902	Art.No. 20N.904	Art.No. 20N.924
Buffer B	10 ml	30 ml	50 ml	270 ml
Proteinase K	1.5 ml	3 ml	4.5 ml	27 ml
Prep Solution	40 ml	100 ml	150 ml	2 x 450 ml
DTT	0.5 ml	1 ml	1.5 ml	9 ml
SDS Solution	11 ml	28	55 ml	280 ml
RNase A	2.5 ml	6 ml	11 ml	60 ml
Lysozyme	37.5 mg (1.5 ml)	112.5 mg (4.5 ml)	150 mg (6 ml)	750 mg (30 ml)
EDTA	1 ml	0.8 ml	2.0 ml	7 ml
nexttec™ cleanPlates96	1	2	4	24
nexttec™ deep-well plate	3	6	12	72
Sealing tapes	3	6	12	72
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.

nexttec GmbH Biotechnologie
Larezhausen 3
86567 Hilgertshausen

Technical Support
Tel: +49 (0) 8250 / 92790 30
Fax: +49 (0) 8250 / 92790 99

www.nexttec.biz
service@nexttec.biz

Storage Conditions

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

Safety Information

Proteinase K 	Danger	H334 P304+P341, P342+P311
DTT 	Warning	H315, H319 P280, P305+P351+P338, P321, P362, P332+P313, P337+P313

Hazard Statements

H315 Causes skin irritation
H319 Causes serious eye irritation
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled

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
P304+P341	IF INHALED: if breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing
P321	Specific treatment (see on this label)
P362	Take off contaminated clothing and wash before reuse
P332+P313	If skin irritation occurs: Get medical advice/attention
P337+P313	If eye irritation persists: Get medical advice/attention

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).

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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 56 °C**
- **Dissolve Lysozyme**
  Add **1.5 ml** (20N.901), **4.5 ml** (20N.902), **6 ml** (20N.904) or **30 ml** (20N.924) of purified **water** to the lyophilized **lysozyme** (final concentration 25 mg/ml).

Protocol

Lysis

L1	Transfer **0.5 ml** of bacterial culture (up to 1.5 OD_{600}) to a deep-well plate. Centrifuge cell suspensions **(2,000 x g, 10 min**), remove and discard the supernatant.
L2	Add **90 µl Buffer B**, **10 µl Lysozyme** and **2.5 µl RNase A*** to the cell pellet. Resuspend cells gently by pipetting up and down. Close the deep-well plate using an alu sealing tape. Incubate with shaking **(56 °C, 200 rpm, 20 min).**
L3	Add **90 µl SDS Solution, 10 µl Proteinase K, 1.5 µl DTT** and **2 µl EDTA*** to each sample. Close the deep-well plate using an alu sealing tape. Incubate with shaking **(56 °C, 200 rpm, 60 min).**

*For Pre-Mixes see Technical Section.

Purification of DNA

P	Transfer **100 µl** of the lysate to an **equilibrated nexttec™ cleanPlate96.** Incubate for **3 min** at **room temperature**. Centrifuge at **700 x g** for **1 min** or apply vacuum for **1 min.**
	The eluate contains the purified DNA!!

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service@nexttec.biz

Technical Section

Preparation of Lysis Pre-Mixes

LB1	**Lysis Buffer LB1**:	1 sample	1 plate	2 plates	4 plates
	Buffer B	90 µl	9.9 ml	19.8 ml	39.6 ml
	Lysozyme	10 µl	1.1 ml	2.2 ml	4.4 ml
	RNase A	20 µl	2.2 ml	4.4 ml	8.8 ml
	Mix by vortexing. Add **120 µl** of **Buffer LB1** to each sample (L2). The **Lysis Buffer LB1** is stable for 1 week if stored at **+2 °C to +8 °C**.				
LB2	**Lysis Buffer LB2**:	1 sample	1 plate	2 plates	4 plates
	SDS Solution	90 µl	9.9 ml	19.8 ml	39.6 ml
	Proteinase K	10 µl	1.1 ml	2.2 ml	4.4 ml
	DTT	2.5 µl	0.27 ml	0.55 ml	1.1 ml
	EDTA	2.0 µl	0.22 ml	0.44 ml	0.88 ml
	Mix by vortexing. Add **100 µl** of **Buffer LB2** to each sample (L3). The **Lysis Buffer LB2** 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.

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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.
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

Web: www.nexttec.biz

Email: service@nexttec.biz

Tel: +49 (0) 8250 / 92 790 32

Fax: +49 (0) 8250 / 92 790 99

Ordering Information

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

Distributor

nexttec GmbH Biotechnologie
Larezhausen 3
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