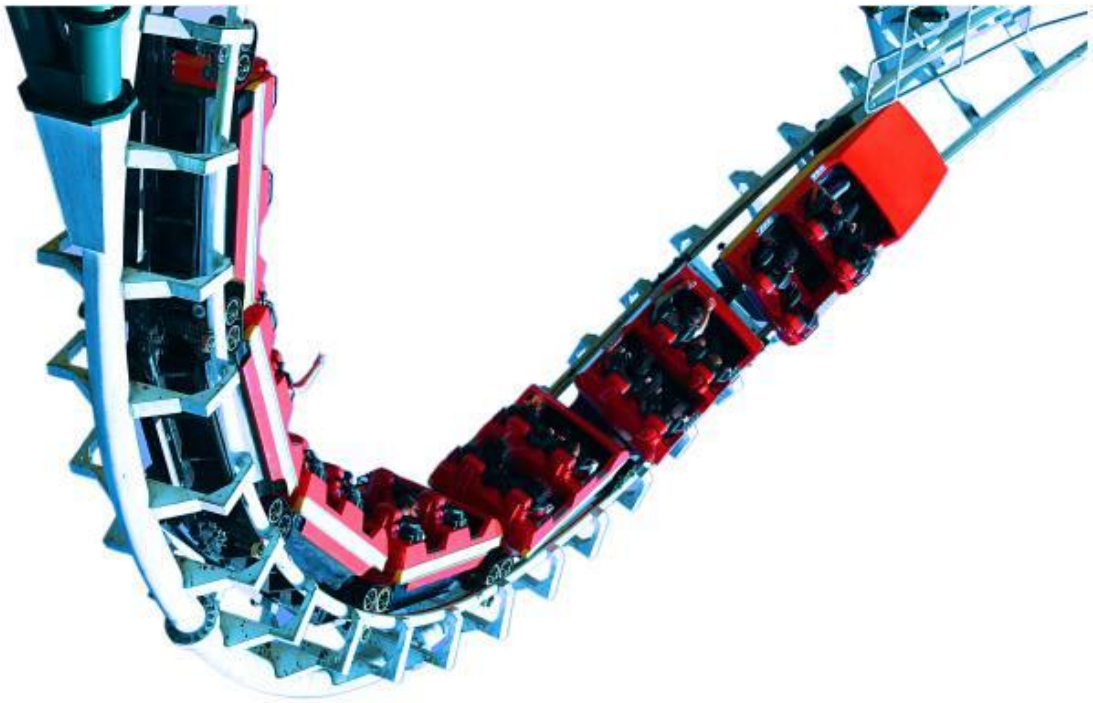

nexttec™

DNA isolation systems

Protocol

DNA Isolation
from Bacteria
by nexttec™ 1-Step

- nexttec™ cleanColumns -

Cat. No. 20N.010	392010N
Cat. No. 20N.050	392020N
Cat. No. 20N.250	392025N

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.010	Art.No. 20N.050	Art.No. 20N.250
Buffer B	2 ml	8 ml	30 ml
SDS-Solution	1.5 ml	7 ml	28 ml
Proteinase K	0.15 ml	0.75 ml	3 ml
Prep Solution	6 ml	20 ml	100 ml
Lysozyme	12.5 mg	25 mg	112.5 mg
	(0.5 ml)	(1 ml)	(4.5 ml)
RNase A	0.5 ml	1.5 ml	6 ml
DTT	0.1 ml	0.2 ml	1 ml
EDTA	0.2 ml	0.5 ml	0.8 ml
nexttec™ cleanColumns	10	50	250
Waste collection tubes	10	50	250
DNA collection tubes	10	50	250

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.

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Storage Conditions

During shipment all kit components are stable at room temperature. After arrival, store **Prep Solution, Buffer B, EDTA, Proteinase K** and **RNase A at +2 °C to +8 °C.** Store **DTT** and **Lysozyme** at **-18 °C to -25 °C.**
SDS-Solution and **nexttec™ cleanColumns** 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

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

P304+P341	IF INHALED: if breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing
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
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™ cleanColumns**

E1	Add **350 µl Prep Solution** to a **nexttec™ cleanColumn**. Incubate for at least **5 min** at room temperature. Centrifuge at **350 x g** for **1 min** to remove excess buffer.
E2	Discard the Waste collection tube. Place the **nexttec™ cleanColumn** into a new DNA collection tube. Use equilibrated nexttec™ cleanColumns or store **closed at +2 °C to +8 °C** and use within **one week.**

- **Preheat a thermomixer to 56 °C**
- **Dissolve Lysosyme**

  Add **0.5 ml** (20N.010), **1.0 ml** (20N.050) or **4.5 ml** (20N.250) of purified **water** to the lyophilized **lysozyme** (final concentration 25 mg/ml).

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Protocol

Lysis

L1	Transfer **0.5 ml of bacterial culture** (up to 1.5 OD_{600}) to a 1.5 ml reaction tube. Centrifuge **(6,000 x g, 1 min)**, remove and discard the supernatant.
L2	Add **90 µl Buffer B**, **10 µl Lysozyme** and **20 µ RNase A*** to the cell pellet. Resuspend cells thoroughly by vortexing. Incubate with shaking **(56 °C, 1200 rpm, 10 min)** in a thermomixer. Incubate with shaking (**60 °C, 1200 rpm, 30 min to overnight**) in a thermomixer
L3	Add **90 µl SDS-Solution, 10 µ Proteinase K, 2.5 µl DTT** and **2 µ EDTA*** to each sample. Vortex and incubate with shaking **(56 °C, 1200 rpm, 30 min)** in a thermomixer.

*For Pre-Mixes see Technical Section.

Purification of DNA

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

Notes:

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Technical Section

Preparation of Lysis Pre-Mixes

LB1	**Lysis Buffer LB1**:	1 sample	<50 samples*	>50 samples*
	Buffer B	90 µl	90 µl x (n+3)	90 µl x (n+5)
	Lysozyme	10 µl	10 µl x (n+3)	10 µl x (n+5)
	RNase	20 µl	20 µl x (n+3)	20 µl x (n+5)
	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	<50 samples*	>50 samples*
	SDS-Solution	90 µl	90 µl x (n+3)	90 µl x (n+5)
	Proteinase K	10 µl	10 µl x (n+3)	10 µl x (n+5)
	DTT	2.5 µl	2.5 µl x (n+3)	2.5 µl x (n+5)
	EDTA	2.0 µl	2.0 µl x (n+3)	2.0 µl x (n+5)
	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**.			

*n= samples [e.g. 22 samples: Buffer B: 90 µl x (22+3)]

- **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.

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- **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™ cleanColumn 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).

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.

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Ordering Information

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

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