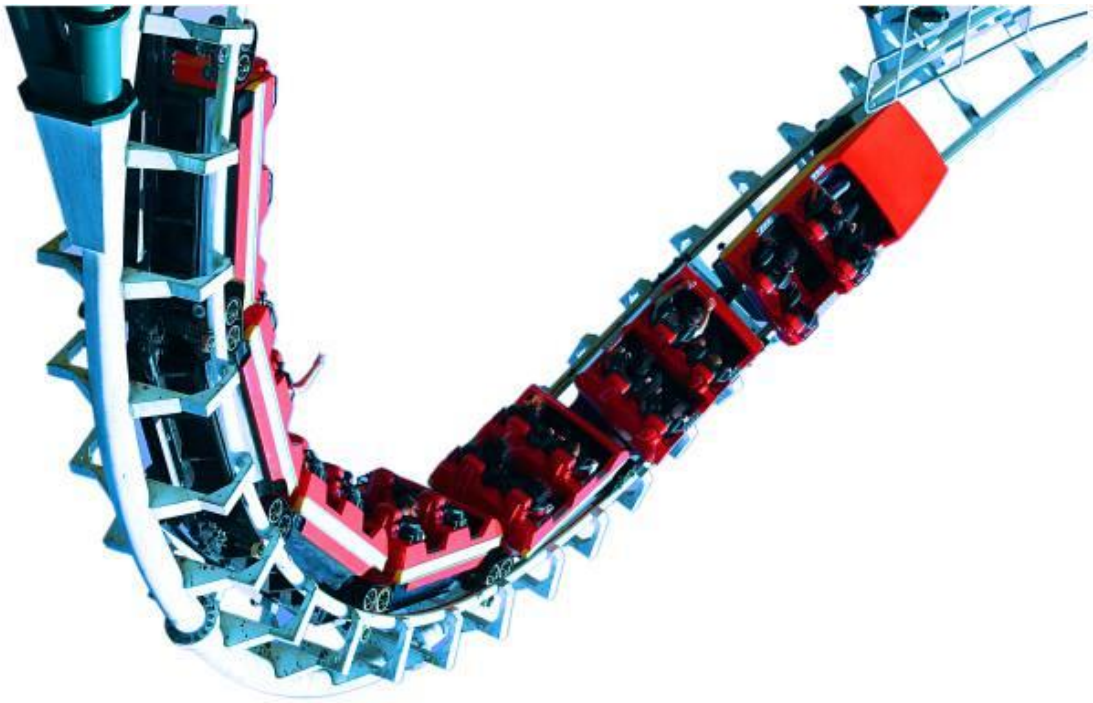

nexttec™

DNA isolation systems

Protocol

DNA Isolation
from Blood (10 µl)
by nexttec™ 1-Step

- nexttec™ cleanColumns -

Cat. No. 55N.010	395510N
Cat. No. 55N.050	395520N
Cat. No. 55N.250	395525N

Version 3.0

For research only

Biozym
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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. 55N.010	Art.No. 55N.050	Art.No. 55N.250
Buffer H	2.1 ml	10.5 ml	42 ml
Proteinase K	0.15 ml	0.75 ml	3 ml
Prep Solution	6 ml	20 ml	100 ml
nexttec™ cleanColumns	10	50	250
Waste collection tubes	10	50	250
DNA collection tubes	10	50	250

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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 **Prep Solution, Buffer H** and **Proteinase K at +2 °C to +8 °C.**
nexttec™ cleanColumns are stored at **room temperature (+20 °C to +25 °C)**. If properly stored, see expiration date for the stability of the kit.

nexttec GmbH Biotechnologie
Larezhausen 3
86567 Hilgertshausen
Germany

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

www.nexttec.biz
service@nexttec.biz

Safety Information

Proteinase K Danger H334

P304+P341, P342+P311

Hazard Statements

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled

Precautionary Statements

P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
P304+P341	IF INHALED: if breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing

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™ 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**

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Protocol

Lysis

L1	Transfer **5-10 µl of EDTA** or **heparinized blood** to a reaction tube.
L2	Add **140 µl Buffer H** and **10 µl Proteinase K*** to each sample. Incubate the sample with shaking **(56 °C, 1200 rpm, 2 h to overnight)** in a thermomixer**.**

*For Pre-Mixes see Technical Section.

Purification of DNA

P	Transfer **80 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:

nexttec GmbH Biotechnologie
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Germany

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service@nexttec.biz

Technical Section

Preparation of Lysis Pre-Mix

LH	**Lysis Buffer LH**:	1 sample	<50 samples*	>50 samples*
	Buffer H	140 µl	140 µl x (n+3)	140 µl x (n+5)
	Proteinase K	10 µl	10 µl x (n+3)	10 µl x (n+5)
	Mix by vortexing. Add **150 µl** of **Buffer LH** to each sample (L2). The **Lysis Buffer LH** is stable for **1 working day** if stored at **+2 °C to +8 °C**.			

*n= samples [e.g. 22 samples: Buffer H: 140 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**.

- **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.

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service@nexttec.biz

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

Biozym
SCIENCE IS OUR BUSINESS
www.biozym.com

Biozym Scientific GmbH
Tel.: 05152 / 9020, Fax: 05152 / 2070
Mail: support@biozym.com

Biozym Biotech Trading GmbH
Tel.: 01 / 334 0156 0, Fax: 01 / 334 0156 88
Mail: support@biozym.com

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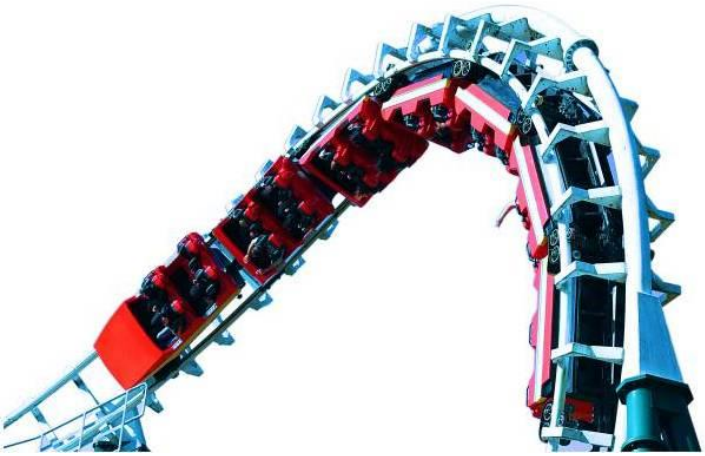

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