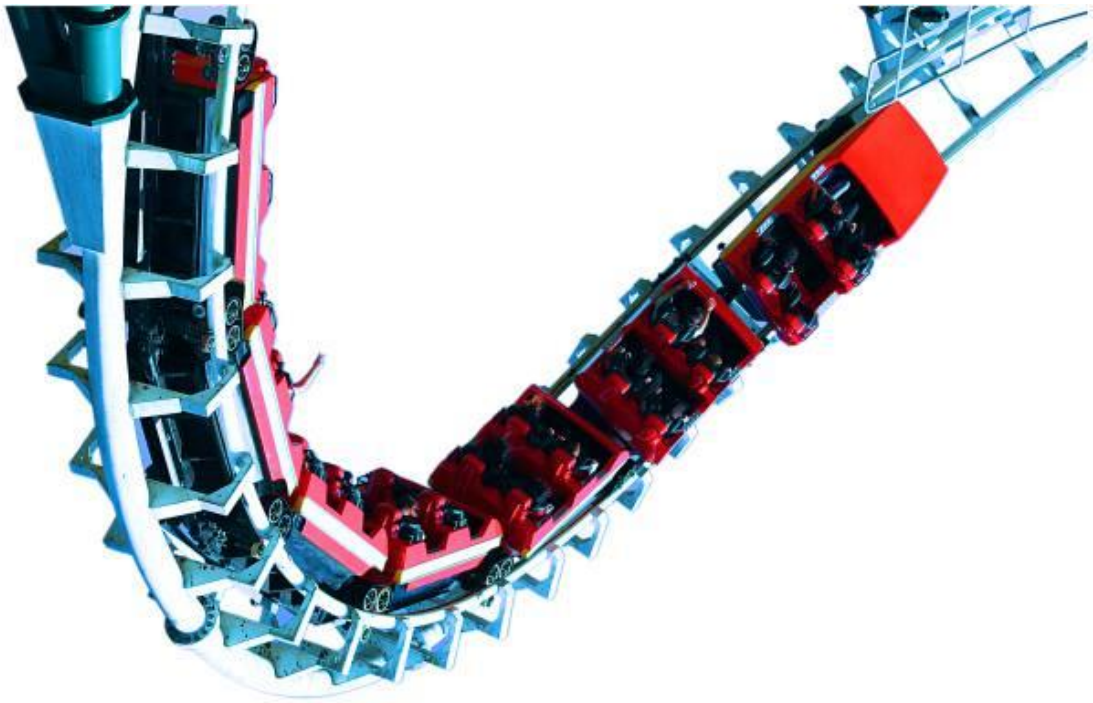

nexttec™

DNA isolation systems

Protocol

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

- nexttec™ cleanColumns -

Cat. No. 30N.010	393010N
Cat. No. 30N.050	393020N
Cat. No. 30N.250	393025N

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.010	Art.No. 30N.050	Art.No. 30N.250
Buffer P	12 ml	12 ml	55 ml
Protease	0.5 ml	0.5 ml	2 ml
Prep Solution	20 ml	20 ml	100 ml
RNase A	1 ml	1.0 ml	5 ml
Lysozyme	12.5 mg	12.5 mg	37.5 mg
	(0.5 ml)	(0.5 ml)	(1.5 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.

Storage Conditions

During shipment all kit components are stable at room temperature. After arrival, store **Prep Solution, Buffer P, Protease** and **RNase A at +2 °C to +8 °C.** Store **Lysozyme** at **-18 °C to -25 °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.

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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

P261	Avoid breathing dust/fume/gas/mist/vapors/spray
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

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 thermostat to 90 °C**
- **Dissolve Lysozyme**
  Add **0.5 ml** (30N.010 and 30N.050) or **1.5 ml** (30N.250) of purified **water** to the lyophilized **lysozyme** (final concentration 25 mg/ml).

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Protocol

Lysis

L1	Transfer **1 ml of overnight bacterial culture** to a reaction tube. Centrifuge (**6,000 x g, 1 min)**, remove and discard the supernatant.
L2	Add **130 µl Buffer P**, **5 µl Protease** and **3 µl Lysozyme*** to the cell pellet. Resuspend cells thoroughly by vortexing or pipetting up and down. Incubate for **5 min at room temperature.**
L3	Transfer the tube to a preheated block thermostat. Incubate at **90 °C for 1 min,** then cool down the solution to room temperature.
L4	Add **15 µl RNase A** to the lysate and 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™ cleanColumn (use 1 ml or 200 µ wide bore pipette tips). 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-Mix

LP	**Lysis Buffer LP**:	1 sample	<50 samples*	>50 samples*
	Buffer P	130 µl	130 µl x (n+3)	130 µl x (n+5)
	Lysozyme	3 µl	3 µl x (n+3)	3 µl x (n+5)
	Protease	5 µl	5 µl x (n+3)	5 µl x (n+5)
	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**.			

*n= samples [e.g. 22 samples: Buffer P: 130 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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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

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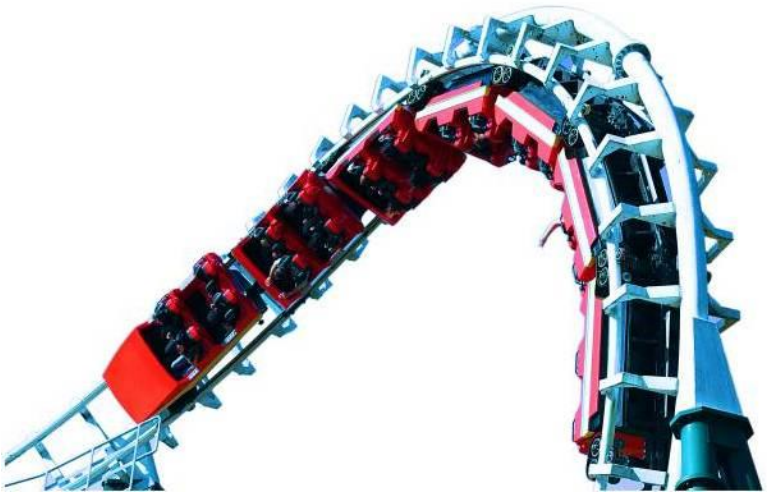

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