



**nexttec**<sup>TM</sup>  
DNA isolation systems

## Protocol

### DNA Isolation from Tissue & Cells by nexttec<sup>TM</sup> 1-Step

- nexttec<sup>TM</sup> cleanColumns -

<b>Cat. No. 10N.010</b>	391010N
<b>Cat. No. 10N.050</b>	391020N
<b>Cat. No. 10N.250</b>	391025N

Version 3.0

**For research only**

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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. 10N.010	Art.No. 10N.050	Art.No. 10N.250
Buffer G	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
DTT (optional for special protocols)	0.1 ml	0.2 ml	1.0 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](mailto:service@nexttec.biz) for detailed information.

**Storage Conditions**

During shipment all kit components are stable at room temperature. After arrival, store **Prep Solution** and **Proteinase K** at **+2 °C to +8 °C**. Store **DTT** at **-18 °C to -25 °C** after first opening.

**Buffer G** and **nexttec™ cleanColumns** 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).

**Before starting**

- **Equilibrate nexttec™ cleanColumns**

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

- **Preheat a thermomixer to 56 °C**

## Protocol

### Lysis

L1	Transfer <b>tissue (5-20 mg fresh weight)</b> or <b>cells (1-2 x 10<sup>6</sup>)</b> to a reaction tube. Centrifuge cell suspensions, remove and discard the supernatant.
L2	Add <b>140 µl Buffer G</b> , <b>10 µl Proteinase K</b> and <i>optional 1.5 µl DTT*</i> to each sample. Incubate the sample with shaking ( <b>56 °C, 1200 rpm, 30 min to overnight</b> ) in a <b>thermomixer</b> .

\*For Pre-Mixes see Technical Section.

### Purification of DNA

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

### Notes:

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## Technical Section

### Preparation of Lysis Pre-Mixes

LG	<b>Lysis Buffer LG:</b>	1 sample	<50 samples*	>50 samples*
	<b>Buffer G</b>	140 µl	140 µl x (n+3)	140 µl x (n+5)
	<b>Proteinase K</b>	10 µl	10 µl x (n+3)	10 µl x (n+5)
	<b>DTT</b>	1.5 µl	1.5 µl x (n+3)	1.5 µl x (n+5)
Mix by vortexing. Add <b>150 µl</b> of <b>Buffer LG</b> to each sample (L2). The <b>Lysis Buffer LG</b> is stable for 1 working day if stored at <b>+2 °C to +8 °C</b> .				

\*n= samples [e.g. 22 samples: Buffer G: 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 =  $299.07 \times \sqrt{350 / r}$  ; 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.

## Contact Information

Web: [www.nexttec.biz](http://www.nexttec.biz)

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## Ordering Information

For ordering information please visit our website [www.nexttec.biz](http://www.nexttec.biz).

## Distributor

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