



nexttecTM

DNA isolation systems

Special Protocol

DNA Isolation

Sperm

by nexttecTM 1-Step

- nexttecTM cleanColumns -

Cat. No. 10N.010 391010N

Cat. No. 10N.050 391020N

Cat. No. 10N.250 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 | 1x 2.1 ml | 1x 10.5 ml | 1x 42 ml |
| Proteinase K | 1x 0,15 ml | 1x 0,75 ml | 1x 3 ml |
| Prep Solution | 1x 6 ml | 1x 20 ml | 1x 100 ml |
| DTT (1,4- Dithio-DL-threitol) | 1x 0.5 ml | 1x 0.5 ml | 1x 0.5 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 time. 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, **Proteinase K solution**, **Buffer G** and **Prep Solution** must be stored at **+2°C to +8°C**.

Store **DTT solution** after first opening at **-18°C to -25°C**. 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

| | |
|-----------------|---|
| 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 350 µl Prep Solution to a nexttec™ cleanColumn . Incubate for at least 5 min at room temperature. Centrifuge at 350x 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**

Protocol

Lysis

| | |
|----|--|
| L1 | Transfer 25 µl sperm to a reaction tube. |
| L2 | Add 140 µl Buffer G , 10 µl Proteinase K and 1,5 µl DTT* to each sample. Incubate the sample with shaking (56°C, 1200 rpm, 3 h) 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 700x g for 1 min . |
| The eluate contains the purified DNA!! | |

Technical Section

- **Preparation of Lysis Pre-Mix**

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

*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 260nm (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

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Ordering Information

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

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