



Special Protocol DNA Isolation *Mouse Tail*

by nexttec™ 1^{-Step}

- nexttec™ cleanPlate96 -

| Cat. No. 10N.901 | 391091N |
|------------------|---------|
| Cat. No. 10N.902 | 391092N |
| Cat. No. 10N.904 | 391094N |
| Cat. No. 10N.924 | 391096N |

Version 3.0



For research only



<u>Principle</u>

nexttecTM 1^{-Step} is the easiest handling and fastest DNA purification system containing a <u>sin-</u><u>gle</u> buffer system and a <u>one-step</u> DNA purification after lysis.

Proteins, detergents and low molecular weight compounds are retained by the nexttec[™] sorbent. DNA passes through the nexttec[™] cleanPlate96 during a short, <u>one-step</u> purification procedure.

The obtained DNA is suitable for all common enzymatic reactions (restriction digests, realtime PCR, PCR, genotyping etc.).

Kit contents

| Component | Art.No. 10N.901 | Art.No. 10N.902 | Art.No. 10N.904 | Art.No. 10N.924 |
|---------------------------------------|--------------------|--------------------|--------------------|--------------------|
| Buffer G | 15 ml | 42 ml | 65 ml | 400 ml |
| Proteinase K | 1.5 ml | 3 ml | 4.5 ml | 27 ml |
| Prep Solution | 40 ml | 100 ml | 150 ml | 2x 450 ml |
| DTT (1,4- Dithio-DL-threitol) | 0.5 ml | 0.5 ml | 1 ml | 5 ml |
| nexttec [™] cleanPlates96 | 1 | 2 | 4 | 24 |
| nexttec [™] deep-well plates | 3 | 6 | 12 | 72 |
| Sealing tapes | 3 | 6 | 12 | 72 |
| Alu sealing tapes | 2 | 4 | 8 | 48 |
| | | 1 | 1 | |

The kit contains all necessary reagents for lysis and subsequent DNA purification.

<u>nexttec™ service</u>

For extending 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, to optimize the lysis time or to use different lysis volumes (e.g. robotic applications).

For detailed information, please get in contact with <u>service@nexttec.biz</u>.

Storage Conditions

During shipment all kit components are stable at room temperature. After arrival, **Proteinase K**, **Buffer G** and **Prep Solution** must be stored **at +2°C to +8°C.** After first opening freeze **DTT** at **-18°C to -25°C.**

nexttec[™] cleanPlates96 can be 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

Proteinase K Danger

H334 P304+P341, P342+P311



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 pro- tection |
|-----------------|--|
| 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. Re- move 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).

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Before starting

• Equilibrate nexttec[™] cleanPlates96

| E1 | Add 350 µl Prep Solution onto each well of a nexttec[™] cleanPlate96 . Incubate for at least 5 min at room temperature and centrifuge at 350x g for 1 min or apply vacuum for 30 to 60 sec to remove excess buffer. |
|----|---|
| E2 | Discard the deep-well plate. Place the nexttec[™] cleanPlate96 onto a new deep-well plate. Use equilibrated nexttec[™] cleanPlates96 or store closed at +2°C to +8°C and use within one week. |

• Preheat an incubator to 56°C

Protocol

| <u>Lysis</u> | | | |
|---------------------------------------|---|--|--|
| L1 | Transfer mouse tails (8 - 12 mg fresh weight; 4-6 mm length) to a deep-well plate. | | |
| L2 | Add 140 µl Buffer G, 10 µl Proteinase K and 1.5 µl DTT* to each sample. | | |
| | Incubate sample with shaking (56°C, 200 rpm, 30 min to overnight). | | |
| *For Pre-Mixes see Technical Section. | | | |
| Purification of DNA | | | |
| | Transfer 100 μI of the lysates to an equilibrated nexttec™ cleanPlate96 . | | |
| | Incubate for 3 min at room temperature . | | |
| P | Centrifuge at 700x g for 1 min or apply vacuum for 1 min . | | |

The eluate contains the purified DNA!!

Notes:

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Technical Section

• Preparation of Lysis Pre-Mixes

| LG | Lysis Buffer LG: | 1 sample | 1 plate | 2 plates | 3 plates | 4 plates |
|----|---|----------|---------|----------|----------|----------|
| | Buffer G | 140 µl | 15.4 ml | 30.8 ml | 46.2 ml | 61.6 ml |
| | Proteinase K | 10 µl | 1.1 ml | 2.2ml | 3.3 ml | 4.4 ml |
| | DTT (optional) | 1.5 µl | 165 µl | 330 µl | 495 µl | 660 µl |
| | Mix by vortexing. Add 150 µl of Buffer LG to each sample (L 2). The Lysis Buffer LG is stable for 1 working day, if stored at +2°C to +8°C . | | | | | |

• 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 concentration with standards, e.g. in ethidium bromide stained agarose gels.

Please notice:

The use of absorption measurement at 260 nm (A₂₆₀) in a spectrophotometer (e.g. NanoDrop[®]) for determination of DNA concentration <u>is system related</u> not recommended. For details and possible workarounds for your specific application please contact: <u>service@nexttec.biz</u>.

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 nexttecTM cleanPlate96 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).

• Vacuum Application

Assemble the vacuum manifold as described in the manual. The flow rate should be between 15 L/min and 150 L/min.

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A regulation of vacuum is not necessary.

Product Use Restriction

nexttecTM 1^{-Step} DNA Isolation Kit components were developed, designed and sold **for re-search 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 nexttecTM 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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