Protocol for Isolation of

Genomic DNA

from Tissue and Cells

by nexttec™ cleanPlates96

Special Protocol:

TypiFix™ Ear Tags

Cat. No. 10.901

Cat. No. 10.902

Cat. No. 10.904

Cat. No. 10.924

Version 4.0

Principle:

As opposed to most other protocols, no DNA is retained by the column resin. Instead proteins, detergents and low molecular weight compounds are retained. DNA passes through the column during a short, one-step purification procedure.
The obtained DNA is suitable for common enzymatic reactions (restriction digests, real-time PCR, PCR, genotyping etc.).

Kit contents

The kit contains all necessary reagents for lysis of Typifx™ ear tag tissue and DNA purification.

Component	Art.No. 10.901	Art.No. 10.902	Art.No. 10.904	Art.No. 10.924
Buffer G1	1x 110 ml	1x 110 ml	1x 150 ml	2x 400 ml
Buffer G2	1x 4.5 ml	1x 4.5 ml	1x 6.0 ml	1x 30 ml
Buffer G3	1x 11 ml	1x 11 ml	1x 14 ml	1x 80 ml
Prep Buffer	1x 20 ml	1x 20 ml	1x 150 ml	2x 450 ml
DTT (1,4- Dithio-DL-threitol)	1x 1.5 ml	1x 1.5 ml	1x 2.0 ml	1x 10 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

Buffer G3	Xn	R 36/38-42;	S 23-24-26-36/37/39
DTT	Xn	R 22-36/37/38;	S 26-36

Risk Phrases

22: Harmful if swallowed; **36/37/38**: Irritating to eyes, respiratory system and skin; **36/38**: Irritating to eyes and skin; **42**: May cause sensitization by inhalation

Safety Phrases

23: do not inhale aerosols; **24**: Avoid contact with skin; **26**: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; **36**: Wear suitable protective clothing; **36/37/39**: Wear suitable protective clothing, gloves and eye/face protection

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Safety Information

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).

Storage Conditions

During shipment all kit components are stable at room temperature. **After arrival, buffer components must be stored at +2℃ to +8℃. After fi rst opening of DTT solution, store remaining DTT at -18℃ to -25℃.**

nexttec™ cleanPlates96 may be stored at room temperature. If properly stored, see expiration date for the stability of the kit.

Determination of DNA concentration in nexttec™ DNA preparations

Absorption measurement cannot be used for DNA preparations obtained by nexttec™ DNA Isolation Kits. Some substances, e.g. buffer components, which are used for sample lysis, remain in the DNA eluate. They cause a higher UV absorption at 260 and at 280 nm and this, in turn, leads to an overestimation of DNA concentration (up to ten-fold) and to a low ratio of A_{260}/A_{280}. This does not influence the quality of DNA for different applications.

For users of nexttec™ DNA Isolation Kits we recommend to determine the DNA concentration by comparing the fluorescence intensity of DNA bands of unknown concentration with that of standards, e.g. in ethidium bromide stained agarose gels.

DNA concentration can also be measured using the fluorescent dye Picogreen®.

For **detailed information** please visit our homepage (**www.nexttec.biz).**

Before starting

- **Equilibrate nexttec™ cleanPlates96**

C1	add **350 µl Prep Buffer** 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
C2	discard the first deep-well plate, place the nexttec™ cleanPlate96 onto a new deep-well plate, store equilibrated nexttec™ cleanPlates96 **closed at +2℃ to +8℃** and use within one week

- **Preheat an incubator with thermostable shaker to 60℃**

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Protocol

Lysis

L1	transfer TypiFix™ ear tag samples to wells of a deep-well plate
L2	add **265 µl Buffer G1**, **10 µl Buffer G2** and **25 µl Buffer G3*** to each sample
	Optional: *to ensure a proper lysis and a high DNA yield add **3 µl of DTT** to each sample*
	close the plate using an Alu sealing tape, incubate with shaking (**60°C, 200 rpm, overnight**) in an incubator
L3	centrifuge the lysate (**2,000x g, 5 min**), take the clear supernatant for DNA purification

*) **For Pre-Mixes see Technical Section.**

Purification of DNA

P1	transfer **120 µl** of the lysates to the **equilibrated** nexttec™ cleanPlate96 and incubate for **3 min** at room temperature
P2	centrifuge at **700x g** for **1 min** or apply vacuum for **1 min**, discard the nexttec™ cleanPlate96 **The eluate contains the purified DNA !!**

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Technical Section

- **Preparation of Lysis buffers (Pre-Mixes)**

LG1	**Lysis Buffer LG1**:	1 sample	1 plate	2 plates	3 plates	4 plates
	Buffer G1	265 µl	31.8 ml	63.6 ml	95.4 ml	127.2 ml
	Buffer G2	10 µl	1.2 ml	2.4 ml	3.6 ml	4.8 ml
	Buffer G3	25 µl	3.0 ml	6.0 ml	9.0 ml	12.0 ml
	DTT (optional)	3 µl	360 µl	720 µl	1.08 ml	1.44 ml
	Mix by vortexing. Add **300 µl of Buffer LG1** to each sample (L2). The Lysis Buffer LG1 is stable for 1 working day if stored **at +2℃ to +8℃** .					

- **Centrifugation**

For centrifugation at 350x g or 700x g use the settings for relative centrifugal force (RCF) of your centrifuge with deep-well plate rotor. Alternatively measure the distance of the nexttec™ cleanPlate96 to the centre of your rotor and calculate the necessary rotations per minute (for example: rpm = 299.07 x $\sqrt{350/r}$; r=radius in cm)

- **Vacuum Application**

Assemble the vacuum manifold as described in the manual. Connect the tube connector to a suitable vacuum source like a water-jet vacuum pump, house vacuum, or membrane vacuum pump (e.g. KNF Model N 035.1.2 A.18). The flow rate should be between 15 L/min and 150 L/min. **A regulation of vacuum is not necessary**.

Load Prep Buffer or samples into the wells of the nexttec™ cleanPlate96. **Incubate for the indicated time (!)** and then switch on the vacuum source. **After the indicated time (!)** switch the vacuum off.

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Product Use Restriction

nexttec™ 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 of clinical use.
It is the responsibility of the user to verify the use of the nexttec™ 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™ products.

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Ordering Information

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