



Special Protocol DNA Isolation

SALIVA

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







Principle

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

Proteins, detergents and low molecular weight compounds are retained by the nexttecTM sorbent. DNA passes through the nexttecTM cleanPlate96 during a short, <u>one-step</u> 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.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	2 x 450 ml
DTT (optional for special protocols)	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

nexttec™ service

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 using different lysis volumes (e.g. robotic applications). For detailed information, please get in contact with **service@nexttec.biz**.

Storage Conditions

During shipment all kit components are stable at room temperature. After arrival, **Proteinase** K and **Prep Solution** must be stored at +2°C to +8°C. Store **DTT** after first opening at -18°C to -25°C. Buffer G and nexttecTM cleanPlates96 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



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DTT (!)

Warning

H315, H319 P280, P305+P351+P338, P321, P362, P332+P313, P337+P313

Hazard Statements

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled

H315 Causes skin irritation

H319 Causes serious eye irritation

Precautionary Statements

P304+P341	IF INHALED: if breathing is difficult, remove victim to fresh air and keep a				
	rest in a position comfortable for breathing				
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER or doc-				
	tor/physician				
P280	Wear protective gloves/protective clothing/eye protection/ face protection				
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes. Remove con-				
P338	tact lenses, if present and easy to do. Continue rinsing				
P338	tact lenses, if present and easy to do. Continue rinsing				
P338	tact lenses, if present and easy to do. Continue rinsing Specific treatment (see on this label)				
P321	Specific treatment (see on this label)				

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[™] 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 350 x g for 1 min or apply vacuum for 30 to 60 sec to remove excess buffer.
E2	Discard the deep-well plate. Place the nexttecTM cleanPlate96 onto a new deep-well plate. Use equilibrated nexttecTM cleanPlates96 or store closed at +2°C to +8°C and use within one week.

Tel: +49 (0) 8250 / 92790 30 Fax: +49 (0) 8250 / 92790 99 Saliva



Preheat an incubator to 56°C

Protocol

<u>Lysis</u>						
	Transfer 1 ml saliva to each well of a deep-well plate. Mix with 4 ml PBS (not in-					
L1	cluded). Centrifuge the mixture (5 min, 2,000 x g, room temperature). Remove					
	and discard the supernatant.					
	Add 140 µl Buffer G, 10 µl Proteinase K and 1.5 µl DTT* to each sample. Dis-					
L2	solve Pellet by vortexing.					
	Incubate samples with shaking (56°C, 200 rpm, 30 min to overnight).					
*For Pre	*For Pre-Mixes see Technical Section.					
<u>Purifica</u>	tion of DNA					
	Transfer 100 μI of the lysates to an equilibrated nexttec™ cleanPlate96.					
	Incubate for 3 min at room temperature.					
Р	Centrifuge at 700 x g for 1 min or apply vacuum for 1 min .					
	The eluate contains the purified DNA!!					

Notes:			



Technical Section

Preparation of Lysis Pre-Mixes

	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		
LG	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 determining 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 (A_{260}) 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: **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 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.

A regulation of vacuum is not necessary.

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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 nexttecTM 1^{-Step} products.

Contact Information

Web: <u>www.nexttec.biz</u>

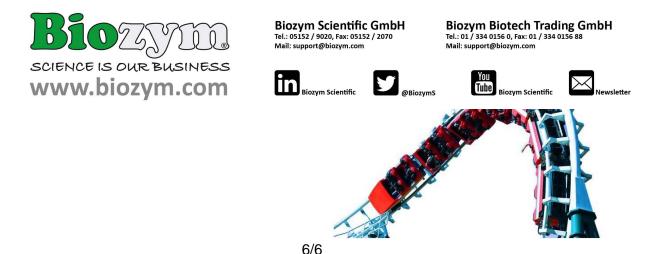
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Ordering Information

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

Distributor



Technical Support

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