XpressBio
Life Science Products

nexttec™
DNA isolation systems

Protocol

DNA Isolation

from Blood (200 µl)

by nexttec™ 1-Step

- nexttec™ cleanPlate96 -

Cat. No. 50N.901

Cat. No. 50N.902

Cat. No. 50N.904

Cat. No. 50N.924

Version 2.0

For research only

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™ cleanPlate96 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. 50N.901	Art.No. 50N.902	Art.No. 50N.904	Art.No. 50N.924
Solution B	2*120 ml	2*250 ml	2*420 ml	10x 500 ml
DTT	0.2 ml	0.5 ml	1 ml	5 ml
Buffer R	14 ml	38 ml	57 ml	344 ml
Proteinase K	2.2 ml	6 ml	9 ml	55 ml
Prep Solution	40 ml	100 ml	150 ml	2x 450 ml
nexttec™ cleanPlates96	1	2	4	24
nexttec™ deep-well plates	3	6	12	72
Sealing tapes	2	4	8	48
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 to use different lysis volumes (e.g. robotic applications).

For detailed information, please get in contact with **xpressbio@xpressbio.com**.

Storage Conditions

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

XpressBio Tel: 301-228-2444 Fax: 301-560-6570 www.xpressbio.com

nexttec™cleanPlates96 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	Xn	R 36/37/38 R42/43;	S 23-24-26 36/37
DTT	Xn	R 22-36/37/38;	S 26-36

Risk Phrases

R 22	Harmful if swallowed
R 36/37/38:	Irritating to eyes, respiratory system and skin.
R 42/43:	May cause sensitisation by inhalation and skin contact.

Safety Phrases

S 23:	Do not breathe Gas/fumes/vapour/spray.
S 24:	Avoid contact with skin.
S 26:	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S 36/37:	Wear suitable protective clothing and gloves.

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 **350x g** for **1 min** or apply vacuum for **30 to 60 sec** to remove excess buffer.

XpressBio Tel: 301-228-2444 Fax: 301-560-6570 www.xpressbio.com

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

Lysis

L1	Transfer **600 µl** cold **Buffer Solution B** to each well of a deep-well plate. Add **200 µl** cold **EDTA or heparinized blood.** Mix thoroughly by pipetting up and down.
L2	Incubate deep-well plate **5 min** on ice. Centrifuge (**2,000x g, 10 min**), remove **700 µl** and discard the supernatant.
L3	Add **500 µl** cold **Buffer Solution B** to each well of a deep-well plate **.** Mix thoroughly by pipetting up and down.
L4	Incubate deep-well plate **5 min** on ice. Centrifuge (**2,000x g, 10 min**), remove **550 µl** and discard the supernatant.
L5	Add **125 µl Buffer R**, **20 µl Proteinase K and 1.5 µl DTT.** Resuspend the pellet by pipetting up and down (3 times). Close the deep-well plate using an alu sealing tape. Incubate with shaking (**56°C, 600 rpm, 30 min**).

*For Pre-Mixes see Technical Section.

Purification of DNA

P	Transfer **100 µl** of the lysates to an **equilibrated nexttec™ cleanPlate96**. Incubate for **3 min** at **room temperature**. Centrifuge at **700x g** for **1 min** or apply vacuum for **1 min**.
	The eluate contains the purified DNA!!

Notes:

XpressBio Tel: 301-228-2444 Fax: 301-560-6570 www.xpressbio.com

Technical Section

- **Preparation of Lysis Pre-Mix**

	Lysis Buffer LR:	1 sample	1 plate	2 plates	3 plates	4 plates
LR	**Buffer R**	125 µl	13.8 ml	27.5 ml	41.3 ml	55 ml
	DTT	1.5 µl	165 µl	330 µl	495 µl	660 µl
	Proteinase K	20 µl	2.2 ml	4.4 ml	6.6 ml	8.8 ml
	Mix by vortexing. Add **146.5 µl** of **Buffer LR** to each sample (L 4). The **Lysis Buffer LR** 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 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:
xpressbio@xpressbio.com

- **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™ 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**

XpressBio Tel: 301-228-2444 Fax: 301-560-6570 www.xpressbio.com

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.

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.

XpressBio Tel: 301-228-2444 Fax: 301-560-6570 www.xpressbio.com

Tel: 301.228.2444
Fax: (301) 560.6570