XpressBio
Life Science Products

nexttec™
DNA isolation systems

Protocol
DNA Isolation
from Plant
by nexttec™ 1-Step

- nexttec™ cleanPlate96 -

Cat. No. 47N.901

Cat. No. 47N.902

Cat. No. 47N.904

Cat. No. 47N.924

Version 1.0

For research only

Principle

nexttec™1^{-Step} is the easiest handling and fasted 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. 47N.901	Art.No. 47N.902	Art.No. 47N.904	Art.No. 47N.924
Buffer F	35 ml	84 ml	150 ml	400 ml
Protease	2.2 ml	6 ml	9 ml	53 ml
Solution H	24 ml	75 ml	90 ml	500 ml
Prep Solution	40 ml	100 ml	150 ml	2x 450 ml
SDS	1.2 ml	3 ml	5 ml	30 ml
DTT (1,4- Dithio-DL-threitol)	0,5 ml	1.5 ml	2 ml	10 ml
RNase A	1.5 ml	4 ml	7 ml	36 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, optimizing 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, store **Buffer F, Prep Solution, Solution H, DTT, Protease and RNase A at +2°C to +8°C.**
SDS and **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.

Safety Information

Protease	Xn	R 36/37/38 R42/43;	S 23-24-26 36/37
DTT	Xn	R 22-36/37/38;	S 26-36
SDS	Xi	R 41;	S 25-26-39

Risk Phrases

R 22:	Harmful if swallowed.
R 42/43:	May cause sensitisation by inhalation and skin contact.
R 36/37/38:	Irritating to eyes, respiratory system and skin.
R 41:	Risk of serious damage to eyes.

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.
S 39:	Wear eye/face protection..

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.
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

A: Without homogenising (using leave pieces)

L1	Transfer **3 -10** pieces of **leaf tissue** (**5 x 5 mm**) to a well of a deep-well plate.
L2	Add **280 µl Buffer F**, and **20 µl Protease*.** Seal plate with an alu sealing tape and incubate (**56°C, 30 min to overnight**).

B: With homogenising

L1	Transfer **50 mg plant leaf tissue** and transfer to a well of a deep-well plate.
L2	Add **185 µl Solution H, 3 µl DTT and 12 µl RNase*** and homogenise tissue in a bead mill (caps for deep-well plates on request).
L3	Add **70 µl Buffer F**, **20 µl Protease and 10 µl SDS.** Close the plate using an Alu sealing tape. Incubate with shaking (**56°C, 750 rpm, 30 min to overnight**).
L4	Centrifuge the lysate (**2,000x g, 3 min**). Use clear supernatant for DNA purification.

C: Plant seeds

L1	Transfer up to **20 mg** seed meal, grist or a single crude squashed seed to a deep-well plate.
L2	Add **280 µl Buffer F**, **20 µl Protease and 3 µl DTT***. Close the plate using an alu sealing tape. Incubate with shaking (**56°C, 750 rpm, 30 min to overnight**).
L3	Centrifuge the lysate (**2,000x g, 3 min**). Use clear supernatant for DNA purification.

*For Pre-Mixes see Technical Section.

<u>Purification of DNA from lysis A,B and C</u>

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!!

<u>Technical Section</u>

Preparation of Lysis buffers (Pre-Mixes)

- **<u>A: without homogenising (using leave pieces)</u>**

LF	**Lysis Buffer LF**:	1 sample	1 plate	2 plates	3 plates	4 plates
	Buffer F	265 µl	31.8 ml	63.6 ml	95.4 ml	127.2 ml
	Protease	10 µl	1.2 ml	2.4 ml	3.6 ml	4.8 ml
	Mix by vortexing. Add **300 µl Buffer LF** to each sample (L2). The **Lysis Buffer LF** is stable for **1** working day if stored **at +2°C to +8°C**.					

- **B: with homogenising**

LF1	**Lysis Buffer LF1**:	1 sample	1 plate	2 plates	3 plates	4 plates
	Solution H	185 µl	22,2 ml	44,4 ml	66,6 ml	88,8 ml
	DTT	3 µl	0,36 ml	0,72 ml	10,8 ml	1,44 ml
	RNase	12 µl	1,44 ml	2,88 ml	4,32 ml	5,76 ml
	Mix by vortexing. Add **200 µl Buffer LF1** to each sample (L2). **Lysis Buffer LF1** is stable for **1** working day if stored **at +2°C to +8°C**.					
LF2	**Lysis Buffer LF2**:	1 sample	1 plate	2 plates	3 plates	4 plates
	Buffer F	65 µl	7,8 ml	15,6 ml	23,4 ml	31,2 ml
	Protease	10 µl	1,2 ml	2,4 ml	3,6 ml	4,8 ml
	SDS	25 µl	3,0 ml	6,0 ml	9,0 ml	12,0 ml
	Mix by vortexing. Add **100 µl Buffer LF2** to each sample (L3). **Lysis Buffer LF2** is stable for **1** working day if stored **at +2°C to +8°C**.					

- **C: for plant seeds**

LF	**Lysis Buffer LF**:	1 sample	1 plate	2 plates	3 plates	4 plates
	Buffer F	265 µl	31.8 ml	63.6 ml	95.4 ml	127.2 ml
	Protease	10 µl	1.2 ml	2.4 ml	3.6 ml	4.8 ml
	DTT	3 µl	360 µl	720 µl	1.08 ml	1.44 ml
	Mix by vortexing. Add **300 µl of Buffer LF1** to each sample (L2). The Lysis Buffer LF1 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 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™ 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.

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 of 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 Life Science Products

503 Gateway Dr W
Thurmont, MD 21788
Tel: 301.228.2444
Fax: (301) 560.6570