XpressBio
Life Science Products

nexttec™
DNA isolation systems

Protocol

DNA Isolation
from Plant
by nexttec™ 1-Step

- nexttec™ cleanColumns -

Cat. No. 47N.010

Cat. No. 47N.050

Cat. No. 47N.250

Version 1.0

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™ cleanColumn 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.010	Art.No. 47N.050	Art.No. 47N.250
Buffer F	4,2 ml	21 ml	84 ml
Protease	0.3 ml	1.5 ml	6 ml
Prep Solution	6 ml	20 ml	100 ml
Solution H	3 ml	15 ml	75 ml
SDS	0.5 ml	0.75 ml	3 ml
DTT (1,4- Dithio-DL-threitol)	0.1 ml	0.25 ml	1.5 ml
RNase A	0.2 ml	1 ml	4 ml
nexttec™ cleanColumns	10	50	250
Waste collection tubes	10	50	250
DNA collection tubes	10	50	250

nexttec™ service

To extend the application range to samples, which is difficult to lyse by the standard procedure, it is recommended to include optional components in the lysis buffer and optimize the lysis conditions. Please get in contact with **service@nexttec.biz** for detailed information.

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™ cleanColumns** are 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™ cleanColumns**

E1	Add **350 µl Prep Solution** to a **nexttec™ cleanColumn**. Incubate for at least **5 min** at room temperature. Centrifuge at **350x g** for **1 min** to remove excess buffer.
E2	Discard the Waste collection tube. Place the **nexttec™ cleanColumn** into a new DNA collection tube. Use equilibrated nexttec™ cleanColumns or store **closed at +2°C to +8°C** and use within **one week.**

- **Preheat a thermomixer to 56°C**

Protocol

Lysis

A: Without homogenising (using leave pieces)

L1	Transfer **3 -10 pieces** of leaf tissue (5x5mm) to a reaction tube.
L2	Add **280 Buffer F**, **20 µl Protease.** Incubate with shaking (**56°C, 1200 rpm, 30 min to overnight**) in a thermomixer

B: With homogenising

L1	Transfer 50 mg plant leaf tissue to a reaction tube.
L2	Add **185 µl Solution H, 3 µl DTT and 12 µl RNase*** and homogenise tissue in a bead mill or by grinding with a pestle.
L3	Add **70 µl Buffer F**, **20 µl Protease** and **10 µl SDS*.** Incubate with shaking (**56°C, 1200 rpm, 30 min**) in a thermomixer.
L4	Centrifuge the lysate (**10,000x g, 1 min**). Use clear supernatant for DNA purification.

C: Plant seeds

L1	Transfer up to **20 mg** seed meal, grist or crude squashed seeds to a reaction tube.
L2	Add **280 µl Buffer F**, **20 µl Protease** and **3 µl DTT*.** Incubate with shaking (**56°C, 1200 rpm, 30 min**) in a thermomixer
L3	Centrifuge the lysate at **10,000x g** for **1 min** Use the clear supernatant for DNA purification.

*For Pre-Mixes see Technical Section.

Purification of DNA from lysis A,B and C

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!!

Technical Section

Preparation of Lysis buffers (Pre-Mixes)

- **A: without homogenising (using leave pieces)**

LF	**Lysis Buffer LF**:	1 sample	<50 samples*	>50 samples*
	Buffer F	280 µl	280 µl x (n+3)	280 µl x (n+5)
	Protease	20 µl	20 µl x (n+3)	20 µl x (n+5)
	Mix by vortexing. Add **300 µl** of **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	<50 samples*	>50 samples*
	Solution H	185 µl	185 µl x (n+3)	185 µl x (n+5)
	DTT	3 µl	3 µl x (n+3)	3 µl x (n+5)
	RNase	12 µl	12 µl x (n+3)	12 µl x (n+5)
	Mix by vortexing. Add **200 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**.			
LF2	**Lysis Buffer LF2**:	1 sample	<50 samples*	>50 samples*
	Buffer F	70 µl	70 µl x (n+3)	70 µl x (n+5)
	Protease	20 µl	20 µl x (n+3)	20 µl x (n+5)
	SDS	10 µl	10 µl x (n+3)	10 µl x (n+5)
	Mix by vortexing. Add **100 µl of Buffer LF2** to each sample (L3). The **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	<50 samples*	>50 samples*
	Buffer F	280 µl	280 µl x (n+3)	280 µl x (n+5)
	Protease	20 µl	20 µl x (n+3)	20 µl x (n+5)
	DTT	3 µl	3 µl x (n+3)	3 µl x (n+5)
	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**.			

*n= samples [e.g. 22 samples: Buffer F: 280 µl x (22+3)]

- **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 260nm (A_{260}) in a spectrophotometer (e.g. NanoDrop®) for determination of DNA concentration is system related not recommend.
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™ cleanColumn 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)

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.

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