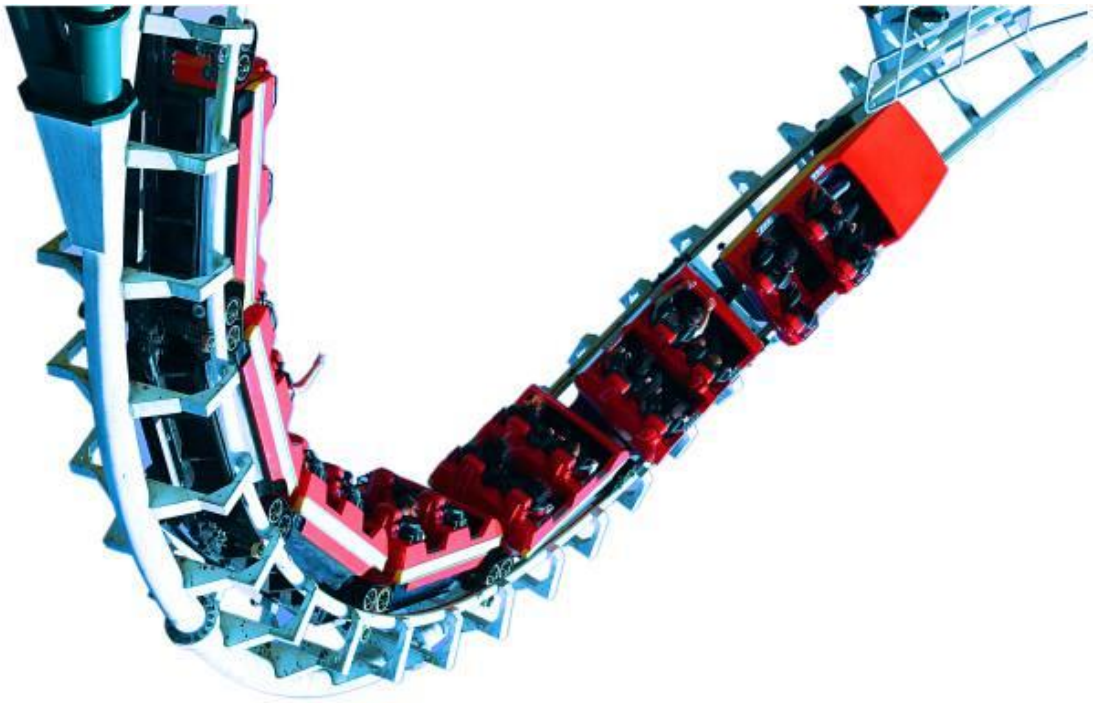

nexttec™

DNA isolation systems

Protocol

DNA Isolation

from Plant

by nexttec™ 1-Step

- nexttec™ cleanPlate96 -

Cat. No. 47N.901	394091N
Cat. No. 47N.902	394092N
Cat. No. 47N.904	394094N
Cat. No. 47N.924	394096N

Version 3.0

For research only

Biozym

SCIENCE IS OUR BUSINESS

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. 47N.901	Art.No. 47N.902	Art.No. 47N.904	Art.No. 47N.924
Buffer F	35 ml	84 ml	150 ml	2x 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	1.0 ml	1.5 ml	9 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, to optimize the lysis time or to use 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 **Prep Solution, Solution H, Protease** and **RNase A at +2 °C to +8 °C.** After first opening freeze **DTT** at **-18 °C to -25 °C. SDS, Buffer F** and **nexttec™ cleanPlates96** are stored at **room**

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temperature (+20 °C to +25 °C). If properly stored, see expiration date for the stability of the kit.

Safety Information

Protease 	Danger	H315, H318, H334, H335 P261, P280, P342+P311, P305+P351+P338
DTT 	Warning	H315, H319 P280, P305+P351+P338, P321, P362, P332+P313, P337+P313
SDS 	Danger	H315, H318 P305+P351+P338

Hazard Statements

H315 Causes skin irritation
H318 Causes serious eye damage
H319 Causes serious eye irritation
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled
H335 May cause respiratory irritation

Precautionary Statements

P261	Avoid breathing dust/fume/gas/mist/vapors/spray
P280	Wear protective gloves/protective clothing/eye protection/ face protection
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
P305+P351+ P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P321	Specific treatment (see on this label)
P362	Take off contaminated clothing and wash before reuse
P332+P313	If skin irritation occurs: Get medical advice/attention
P337+P313	If eye irritation persists: Get medical advice/attention

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).

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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 **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 60 °C**

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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 (**60 °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 (**60 °C, 750 rpm, 30 min to overnight**).
L4	Centrifuge the lysate (**2,000 x 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 (**60 °C, 750 rpm, 30 min**).
L3	Centrifuge the lysate (**2,000 x g, 3 min**). Use 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™ cleanPlate96. Incubate for **3 min** at **room temperature**. Centrifuge at **700 x g** for **1 min.**
	The eluate contains the purified DNA!!

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Technical Section

Preparation of Lysis buffers (Pre-Mixes)

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

LF	**Lysis Buffer LF**:	1 sample	1 plate	2 plates	3 plates	4 plates
	Buffer F	280 µl	30.8 ml	61.6 ml	92.4 ml	123.2 ml
	Protease	20 µl	2.2 ml	4.4 ml	6.6 ml	8.8 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	20.4 ml	40.7 ml	61.2 ml	81.4 ml
	DTT	3 µl	0.33 ml	0.66 ml	0.99 ml	1.32 ml
	RNase	12 µl	1.32 ml	2.64 ml	3.96 ml	5.28 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	70 µl	7.7 ml	15.4 ml	23.1 ml	30.8 ml
	Protease	20 µl	2.2 ml	4.4 ml	6.6 ml	8.8 ml
	SDS	10 µl	1.1 ml	2.2 ml	3.3 ml	4.4 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	280 µl	30.8 ml	61.6 ml	92.4 ml	123.2 ml
	Protease	20 µl	2.2 ml	4.4 ml	6.6 ml	8.8 ml
	DTT	3 µl	0.33 ml	0.66 ml	0.99 ml	1.32 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**.					

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- **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 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 350 x g or 700 x 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.

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Ordering Information

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