Special Protocol

DNA Isolation from

Bacteria in Milk

by nexttec™ 1-Step

- nexttec™ cleanColumns -

Cat. No. 22N.010

Cat. No. 22N.050

Cat. No. 22N.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. 22N.010	Art.No. 22N.050	Art.No. 22N.250
Buffer B	2 ml	8 ml	30 ml
Solution S	3.5 ml	3.5 ml	15 ml
SDS	1.5 ml	7 ml	28 ml
Proteinase K	0.15 ml	0.75 ml	3 ml
Prep Solution	6 ml	20 ml	100 ml
RNase A	0.3 ml	1.5 ml	6 ml
Lysozyme	12.5 mg	25 mg	112.5 mg
DTT	0.1 ml	0.2 ml	1 ml
EDTA	0.2 ml	0.5 ml	0.8 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 are difficult to lyse by the standard procedure, it is recommended to include optional components in the lysis buffer and optimize the lysis time. 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, **Proteinase K, Lysozyme, Solution S, Buffer B, SDS, Prep Solution** and **RNase A** must be stored at **+2°C to +8°C.**
nexttec™ cleanColumns 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	Xn	R 36/37/38 R42/43;	S 23-24-26 36/37
Solution S	Xn	R 36/38-42;	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™ 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.**

- **Dissolve Lysozyme**

  Add **1.0 ml** (22N.010 / 22N.050) or **4.5 ml** (22N.250) of purified **water** to the lyophilized **lysozyme** (final concentration 25 mg/ml).
  Mix thoroughly by vortexing and store at **-18°C to -25°C.**
- **Preheat a thermomixer to 56°C**

Protocol

Lysis

L1	Transfer **1 ml** of **fresh unfiltered milk** samples to a reaction tube.
L2	Add **40 µl of Solution S** to each milk sample. Mix by vortexing and incubate with shaking (**60°C, 1200 rpm, 5 min**).
L3	Centrifuge samples (**3 min, 13,000x g**), Vacuum the fatty top layer and the clear supernatant using a water-operated vacuum pump**
L4	Add **90 µl Buffer B**, **10 µl Lysozyme** and **20 µl RNase A*** to the cell pellet. Resuspend cells by thorough vortexing Incubate with shaking (**60°C, 1200 rpm, 10 min**).
L5	Add **90 µl SDS, 10 µl Buffer Proteinase K, 2.5 µl DTT and 2 µl EDTA*** to each sample Vortex and incubate with shaking (**56°C, 1200 rpm, 30 min**) in a thermomixer.

*For Pre-Mixes see Technical Section.

** see Technical Section “Removal of fatty supernatant”

Purification of DNA

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!!

Notes:

Technical Section

- **Preparation of Lysis buffers (Pre-Mixes)**

LB1	**Lysis Buffer LB1**:	1 sample	<50 samples*	>50 samples*
	Buffer B	90 µl	90 µl x (n+3)	90 µl x (n+5)
	Lysozyme	10 µl	10 µl x (n+3)	10 µl x (n+5)
	RNase A	20 µl	20 µl x (n+3)	20 µl x (n+5)
	Mix by vortexing. Add **120 µl of Buffer LB1** to each sample (L4). **Buffer LB1** is stable for **1** week if stored at **+2°C to +8°C**			
LB2	**Lysis Buffer LB2**:	1 sample	<50 samples*	>50 samples*
	SDS Solution	90 µl	90 µl x (n+3)	90 µl x (n+5)
	Proteinase K	10 µl	10 µl x (n+3)	10 µl x (n+5)
	DTT	2.5 µl	2.5 µl x (n+3)	2.5 µl x (n+5)
	EDTA	2.0 µl	2.0 µl x (n+3)	2.0 µl x (n+5)
	Mix thoroughly by vortexing. Add **100 µl Buffer LB2** to each sample (L5). Lysis Buffer LB2 is stable for **1** working day if stored **at +2°C to +8°C**.			

*n= samples [e.g. 22 samples: Buffer B: 90 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).

- **Removal of fatty supernatant (L3)**

Remove the fatty top layer as completely as possible. Do not to lose the fine cell pellet. Alternatively, the cell pellet can be washed 2 times with TBS buffer (10 mM Tris-HCl; 150 mM NaCl; pH 8.0).

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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