BIORON life science

PureColumn DNA Extraction Kit

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Kit for isolation of DNA from canned and high processed food or other products for subsequent detection of DNA

Research Use Only (RUO)

Content

PureColumn DNA Extraction Kit	Cat. No.: BI88050 (50 preps)
	Cat. No.: BI88100 (5x 50 preps)
Valid from:	May 2020

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1. Introduction

PureColumn DNA Extraction Kit is designed for an efficient and fast preparation of pure DNA. The kit contains spin columns, buffers and reagents necessary for cell lysis, DNA binding, washing and elution of DNA into small volume. Each kit contains a manual with detailed protocols of DNA extraction and purification from whole blood, cells and food.

PureColumn DNA Extraction Kit offers a timesaving, easy and high yield DNA purification system. The procedure is based on optimized buffers and the use of specially designed columns. The advanced buffer system is used for efficient recovery of DNA and removal of contaminants. DNA is adsorbed to the uniquely designed spin membrane and all impurities are efficiently removed by washing. The pure DNA is directly eluted in a special buffer.

DNA is suitable for downstream applications including PCR, hybridization, restriction analysis, cloning and other. The quality of DNA according to spectrophotometry holds the standard for pure DNA solutions. The A_{260}/A_{280} ratio of DNA extracted with the PureColumn DNA Extraction Kit shows values between 1.7 and 2.0.

Samples

Blood (fresh or frozen) samples, with or without anti-coagulating agents (e.g. EDTA, heparin, citrate), plasma and other body liquid or up to 10^7 cells (grown in suspension or adherent) to isolate genomic DNA.

For isolating genomic DNA for **species detection** or for isolating of genomic DNA from **bacteria (pathogen diagnostic):** food samples (processed and preserved), milk and milk products, sausages, salami, meat and cheese.

2. Content of the Kit

Ref No	SBI88050 10 preps (sample size)	BI88050 50 preps	BI88100 2 x 50 preps	Storage
Mini spin columns	10	50	2 x 50	RT
Collection tubes 2.0 ml	10	50	2 x 50	RT
Lysis Buffer TCBL-1 [a]	11 ml	11 ml	2 x 11 ml	RT
Binding Buffer TGP-2	11 ml	11 ml	2 x 11 ml	RT
Wash Buffer TGI-3 concentrate [b]	18 ml	18 ml	2 x 18 ml	RT
Wash Buffer TE-4 concentrate [c]	5.1 ml	2x 5.1 ml	4 x 5.1 ml	RT
Elution Buffer EL-3	11 ml	11 ml	2 x 11 ml	RT
Proteinase K (20 mg/ml)	1 ml	1 ml	2 x 1 ml	-20°C
Manual	1	1	1	1

a Precipitates in Lysis Buffer TCBL-1 should be dissolved by warming up to 55 °C**.**

b / **c Note before starting:**
Preparation of Wash buffer TGI-3 and Wash Buffer TE-4 (see page 4)

3. Storage Conditions and Stability

Spin columns of the kit are packed in closed bags and show full performance in this condition at room temperature (18 - 25 °) for at least 2 years. Proteinase K is delivered as solution and should be stored upon arrival at -20 C. Store buffers at room temperature (18 - 25 °C). Keep all components of the kit away from direct sunlight.

Guarantee for full performance of the kit as specified in this manual is only valid if storage conditions are followed. Please take care that columns, once opened, should be used instantly.

4. Quality Control

The performance of the **PureColumn DNA Extraction Kit** is monitored routinely on a lot-to-lot basis.

5. Safety Information

The following components of **PureColumn DNA Extraction Kit** contain hazardous contents. It is strongly recommended to wear a lab coat, disposable gloves and protective goggles when working with chemicals. More detailed information is available in the material safety data sheet, which can be requested from the manufacturer. There is no need of labeling harmful features with H & P phrases upon packing sizes of 125 ml or 125 g.

Component	Hazard content	GHS symbol		Hazard phrases	Precaution phrases
Lysis Buffer TCBL-1	SDS Sodium dodecyl sulphate (0.1-1%)		Warning	302, 319	280, 301+312 305+351+338, 330, 337+313
Binding Buffer TGP-2	Guanidine hydrochloride 36-50%		Warning	302, 319	280, 301+312 305+351+338, 330, 337+313
Wash Buffer TGI-3	Guanidine hydrochloride 36-50%		Warning	302, 319	280, 301+312 305+351+338, 330, 337+313
Proteinase K Solution	Proteinase K 20 mg/ml	 	Warning Danger	315, 319, 334, 335	261,280, 302+352, 304+340, 305+351+338,312, 332+313, 337+313, 342+311, 403+233

Hazard phrases	
H302	Harmful if swallowed
H315	Cases skin irritation
H319	Causes serious eye irritation
H334	May cause allergy asthma symptoms or breathing difficulties if inhaled
H335	May cause respiratory irritation

Precaution phrases	
P280	Wear protective gloves / eye protection
P301+312	If swallowed: call a poison center/doctor/ …/ if you well unwell
P302+352	If on skin: wash with plenty of water
P304+340	If inhaled: go to fresh air and keep at rest in a position comfortable for breathing
P305+351+338	If in eyes: rinse cautiously with clean water for several minutes. Remove contact lenses. Continue rinsing
P312	Call a poison center/doctor/../ if you feel unwell
P330	Rinse mouth
P332+313	If skin irritation occurs: get medical advice/ attention
P337+313	If eye irritation persists: get medical advice/attention
P342+311	If experiencing respiratory symptoms: call a poison center/ doctor
P403+233	Store in a well ventilated place. Keep container tightly closed

6. Procedure for extraction and DNA purification

Additional material required:

- 96-100 % ethanol
- 100 % isopropanol
- Incubator/ heat shaker or water bath
- Microcentrifuge
- Receiver tubes (1.5 ml)

Before starting:
Wash Buffer TGI-3 and Wash Buffer TE-4 are concentrates. Before using for the first time, add the appropriate amount of isopropanol (100 %) (Wash Buffer TGI-3) and ethanol (Wash Buffer TE-4) as indicated on the bottle and in the table below:

Add the appropriate amount of alcohol and mix well:

Ref. No.	**SBI88050** 10 preps	**BI88050** 50 preps
Wash Buffer TGI-3 concentrate	**Add 7.6 ml isopropanol** to 18 ml	**Add 7.6 ml isopropanol** to 18 ml
Wash Buffer TE-4 concentrate	**Add 21 ml ethanol** to 5.1 ml	**Add 21 ml ethanol** to 5.1 ml

Required incubators /water baths
Heat an incubator up to 55 °C (see step 1 in protocol) and another to 70 °C (see step 3). Heat Elution Buffer EL-3 up to 70 °C for elution (see step 12).

6a. Pre-treatment of samples

Recommended liquid sample volume:

- Blood, serum, milk or other liquid sample: 200 µl (direct, without centrifugation)
- Cells: 200 µl (10^6-10^7 cells/ml)

Concentration of liquid samples by centrifugation:

- For isolation of genomic DNA from bacteria in milk or other non-viscous samples cells can be pelleted by centrifugation for 10 minutes at 5000 g.
- Discard the supernatant and resuspend the pellet in a 1.5ml tube with 200 µl Lysis Buffer for DNA extraction, see step 1 in protocol.

Sample enrichment by cultivation:

- For enrichment of liquid media containing bacteria, liquid sample is transferred into tryptose soy bean media (not provided).
- Liquid media is incubated for 1-2 days and 200 µl sample is taken out in 1.5 ml tube for lysis (see step one in protocol) or for concentration of the sample by centrifugation (see above).

Solid food or tissue sample:

- Solid food sample: 50 mg (meat, sausage, salami). Grind the sample with a mini scalpel or pulverize the sample in liquid nitrogen or use a mini mortar/pistil or mini homogenizer.
- Soft food sample can be lysed with the lysis buffer directly (see step 1 of protocol).
- Complex solid samples like cornflakes must be sheared or crushed with a mini homogenizer or sterile mini pistil.

Solid food contaminated with bacteria:

- About 50 mg food sample contaminated with bacteria (most of bacteria are on surface of food samples) can be enriched by incubation of food sample in specific nutrient media (about 2 ml - 5 ml).
- After overnight incubation with shaking (rotator or thermal shaker) at 37°C about 200 µl nutrient media are transferred into a 1.5ml tube for lysis (see step 1 in protocol).
- Alternatively, to increase sensitivity, about 400 - 800 µl of enriched media are centrifuged for 10 minutes at 5000 g and pellet is resuspended in a 1.5 ml tube with 200 µl Lysis Buffer for DNA extraction (see step lysis). Proceed with DNA extraction according to protocol.

Gram-positive bacteria:

- Gram-positive bacteria need a lysozyme treatment prior lysis step (available on request)
- Take an aliquot of 100 µl of enriched bacteria solution or 500 µl of non-enriched liquid bacteria sample
- Centrifuge for 5 minutes at 5000 g and discard supernatant
- Resuspend pellet with 100 µl Resuspension Buffer L
- Add 10 µl Lysozyme (20 mg/ml) and incubate 30 minutes at 37 °C

6b. Extraction protocol

Pre-step for gram-positive bacteria: treatment with lysozyme, see page 6

1. Add **200 µl Lysis Buffer TCBL-1** + **20 µl Proteinase K Solution** (20mg/ml) to the sample. Vortex thoroughly at maximum speed for 1 minute.
2. Incubate the tube at 55 °C in heat shaker for 60 minutes.
3. Add **200 µl Binding Buffer TGP-2** to lysate and vortex for 10 seconds and incubate the solution at 70°C for 10 minutes.
4. Centrifuge the tube at 13000 rpm (approx. 10000 g) for 5 minutes and transfer the DNA-containing supernatant to a new tube.
5. Add **200 µl ethanol** to DNA solution and vortex for 10 seconds.
6. Place a spin column in a provided 2 ml collection tube. Apply the **600 µl liquid sample** to the column and centrifuge the tube at 10000 rpm (approx.7400 g) for 1 minute.
7. Discard flow through. Place the column back into the same tube. Collection tubes are re-used to reduce plastic waste.
8. Add **500 µl Wash Buffer TGI-3** (add isopropanol before use, as indicated on the bottle) to the column and centrifuge at 10000 rpm (approx. 7400 g) for 1 minute.
9. Discard flow through. Place the column back into the collection tube.
10. Add **500 µl Wash Buffer TE-4** (add ethanol before use as indicated on the bottle) to the column and centrifuge at 10000 rpm (approx. 7400 g) for 1 minute
11. Repeat step 10 one time, but centrifuge for 2 minutes at 10000 rpm to remove complete wash buffer (residue of wash buffer can decrease the DNA recovery).
12. Place the column in a 1.5 ml receiver tube (not provided) and heat up the column at 70°C for 2 - 5 minutes to remove residues of alcohol. Add **50-100 µl Elution Buffer EL-3** (heated up to 70°C) to the column and incubate for 2 minutes at room temperature.
13. Centrifuge at 10000 rpm (approx. 7400 g) for 1 minute and collect the eluate and proceed with down-stream processing (gel electrophoresis, PCR etc.).

Note: it is very important to remove rests of alcohol. Please follow the instructions in step 11 and 12!

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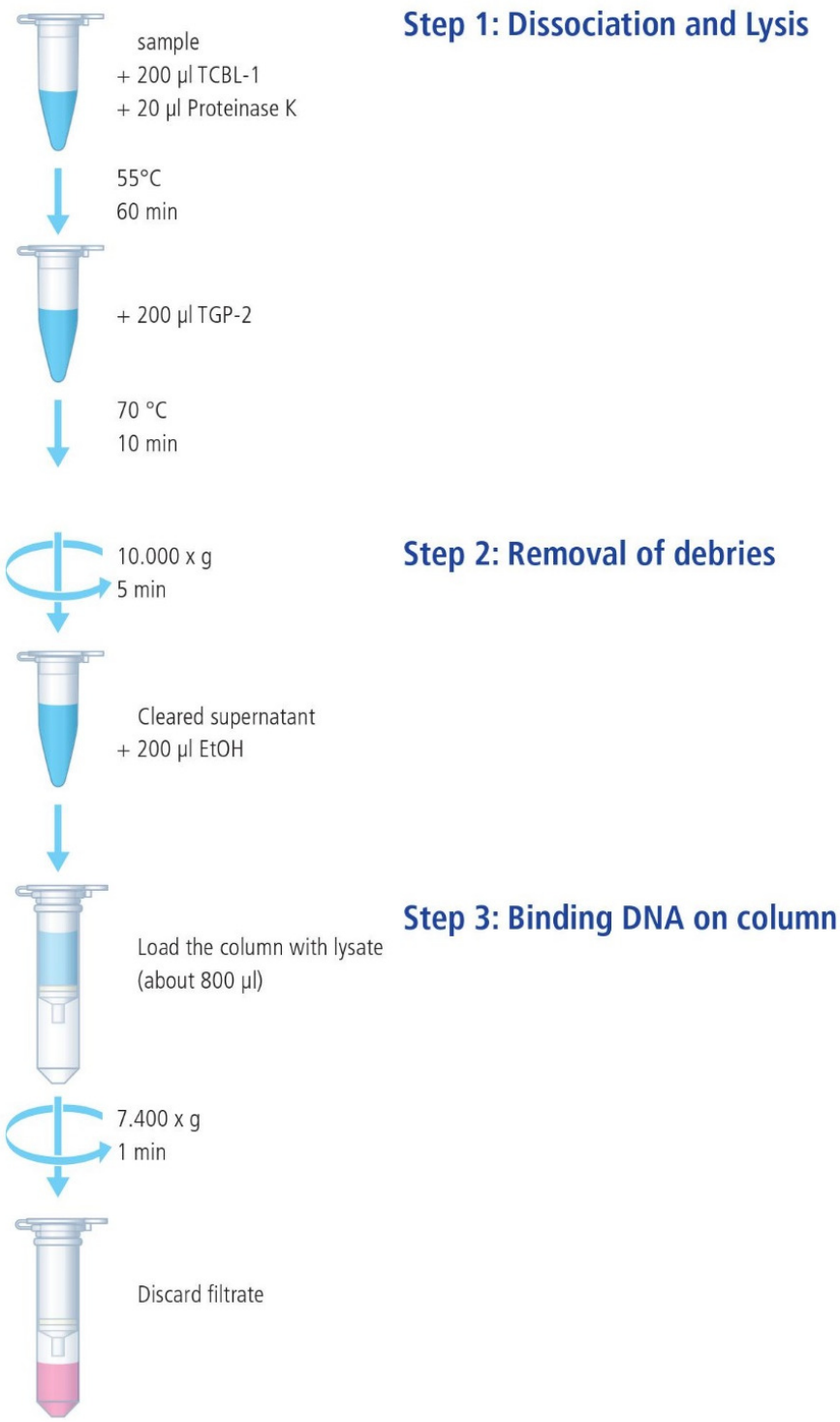
7. Flowchart of Extraction



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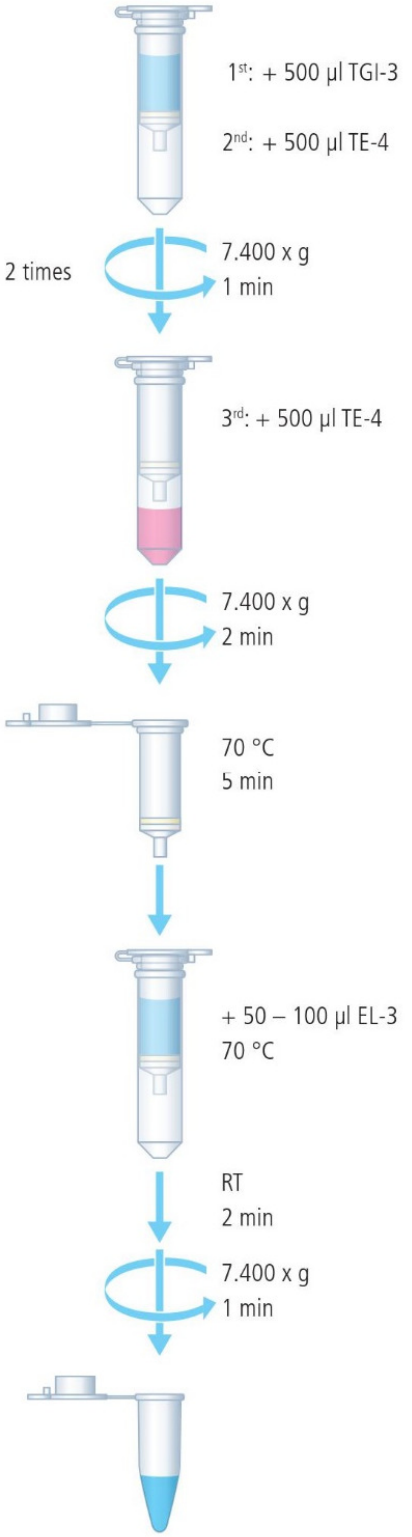

Step 4: Wash the column

Step 5: Elution of DNA

7. Troubleshooting

This guide can help to solve problems that may arise.

Observation	Possible cause	Comments/suggestions
Low or no DNA yield	Inefficient lysis of sample Lysis Buffer TCBL-1 not added Binding Buffer TGP-2 not added	Proteinase K added to Lysis Buffer? Stored at -20°C? Ensure that Lysis Buffer TCBL-1 has been added and mixed with the lysate. Ensure that Binding Buffer TGP-2 has been added to and mixed with the lysate
DNA “smear”	Nuclease activity/ contamination	Upon disintegration of samples, cellular nucleases are released and may degrade genomic DNA. Whenever possible, fresh samples should be used and processed immediately. Use only sterilized glass and plastic ware in order to avoid nuclease contamination.
Low DNA performance	Salt in eluate	Make sure that you followed all washing steps of the procedure.

8. Warranty and Guarantee of Products

The manufacturer guarantees the performance of its PureColumn DNA Extraction Kit in the manner described in this handbook. It is up to the user to determine the suitability of PureColumn DNA Extraction Kit for its particular use. In case a product fails to perform due to any reason except misuse, the manufacturer will replace it without further charge or refund the purchase price. We reserve the right to change, alter or modify our PureColumn DNA Extraction Kit to enhance its performance and design. The manufacturer’s terms and conditions are available on request.

9. Limitations of Product Use

The use of all contents of **PureColumn DNA Extraction Kit** is strictly limited to research purposes. The contents of this kit are not to be applied for any diagnostic use, including human or drug purposes.