Manufactured by:

BIOTOOLS B&M Labs, S.A.
Valle de Tobalina - 52 - Nave 39
28021 Madrid
Spain

Tel. (34) 91 710 00 74
Fax (34) 91 505 31 18
e-mail: info@biotools.eu
www.biotools.eu

SPEEDTOOLS FOOD DNA EXTRACTION KIT

Designed for the rapid isolation of highly pure genomic DNA from food samples of plant or animal origin

Instructions for Use

(Cat. No. 21.176/7)

PLEASE READ THE INSTRUCTIONS FOR USE THOROUGHLY BEFORE USING THE KIT, ESPECIALLY IF YOU ARE NOT FAMILIAR WITH THE PROTOCOL.

SPEEDTOOLS FOOD DNA EXTRACTION KIT

1. BASIC PRINCIPLE

SPEEDTOOLS FOOD DNA EXTRACTION KIT is designed for the rapid isolation of highly pure genomic DNA from **food samples of plant or animal origin**.

Food samples due to their composition (additives, fat, colourings, preservatives) are very heterogeneous samples containing different compounds which can lead to suboptimal extraction or subsequent processing of DNA. Therefore, it is a must that the DNA purification method eliminate these inhibitors, keeping DNA integrity. Speedtools Food DNA Extraction Kit guarantees a good recovery for small genomic DNA fragments (<1 kb) out of processed or complex food matrices (e.g. ketchup or spices), which generally have low DNA concentrations as well as poor quality/degraded DNA.

To start the protocol food samples must be homogenised, the DNA is extracted with Proteinase K and lysis buffer (Buffer BCF/Proteinase K) containing chaotropic salts, denaturing agents and detergents. Lysis mixtures should be cleared by centrifugation in order to remove contaminants and residual cellular debris. The clear supernatant is then mixed with the binding buffer (Buffer BC4) and ethanol to create conditions for optimal DNA binding to the silica membrane of the column. After washing with two different buffers (Buffer BCQW and Buffer BC5) for efficient removal of potential PCR inhibitors, DNA can be eluted in low salt buffer (Buffer BCE) or water, and is ready-to-use in subsequent reactions.

2. KIT CONTENTS

REAGENTS	***50 Preps Cat. No. 21.176***
Buffer BCF	100 ml
Buffer BC2	24 ml
Buffer BC3	6 ml
Buffer BC5 (concentrate)	20 ml
Buffer BCE	15 ml
Buffer BCQW	30 ml
Proteinase K (lyophilized)	6 mg
Proteinase Buffer	0.8 ml
Speedtools Food Columns	50
Protocol	1
Collecting Tubes for the column	150+50

3. INTENDED USE

With the SPEEDTOOLS FOOD DNA EXTRACTION method genomic **DNA is purified from food samples preferably of plant or animal origin.** The protocol should be started with up to 200 mg of material in processed food due to the low DNA content of the samples.

The Kit can be used for the **purification of GMO-DNA** and the **purification of DNA from animal origin in food and feed samples**.

Bacteria DNA in food samples can also be processed, for this purpose we recommend an overnight preculture of the sample in an appropriate culture medium. Centrifuge an aliquot of the culture and start the preparation with the bacterial pellet.

Lysis Buffer BCF have been tested for extraction of DNA from various types of samples (see the following table). Some samples may require to adapt the standard protocol, please contact to our Technical Dept. (info@biotools.eu).

Table 1. Positive Tested Samples

FOOD from plant origin	• Raw products: maize, soya, rape, etc. (powder or oil*) • Chocolate products, cocoa, nougat products • Breakfast cereals, muesli, nut/chocolate spread • Jam and fruit concentrates • Cookies, cakes and biscuits • Pollen • Lecithin • Spices* • Bread
FOOD from animal origin	• Raw and processed products (meat, sausage, pie)
PHARMACEUTICALS	• Plant (starch) compounds in pharmaceuticals (e.g. tablets) • Vitamins (e.g. pills)
COSMETICS	• Plant and animal ingredients in crème or powder
BACTERIA	• Starter Cultures

*Standard protocol must be adapted contact our Technical Dept. (info@biotools.eu)

Fluid samples such as ketchup, sauce and similar (200 mg equivalents) can be mixed with lysis Buffer BCF (500-1000 µl each) and incubated with Proteinase K as described in the protocol.

For **powdered hygroscopic samples**, more lysis Buffer BCF than the indicated in the protocol can be used, lysis solution should be semi fluid and it can be pipetted. Extraction can be improved by preincubation of sample with lysis Buffer BCF for 1-2 hours.

Standard procedure allows processing of up to 200 mg material. Depending on the individual sample, typical yields for the Kit are in the range of 0.1-10 µg DNA. The obtained DNA is ready-for-use in subsequent reactions like PCR, real-time PCR, GMO detection etc.

Table 2. General Characteristics of the Kit

	Food
Sample Size	Up to 200 mg
Average Yield	0.1-10 µg
Elution Volume	100 µl
Binding Capacity	30 µg
Time / Prep	35 min/ 6 prep
Size of DNA	>300 bp
Column Type	mini

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Wear a lab coat, disposable gloves, and protective goggles.

- Appropriate equipment for sample homogenization if required e.g. mortar and pestle, commercial homogenizers, steel beads, etc.
- Microcentrifuge
- Water bath/Incubator
- Vortex
- Ethanol 96-100%
- Microcentrifuge tubes (1.5 ml and 2 ml)
- RNase solution (20 mg/ml)

5. PREPARATION OF WORKING SOLUTIONS AND STORAGE CONDITIONS

NOTE

***Buffers BC2, BC3, BC4 and BCQW contain guanidine hydrochloride and detergents therefore wear gloves and goggles**.*

All kit components can be stored at room temperature (20-25°C) and are stable up to one year.

Before starting any protocol prepare the following reagents:

- **Proteinase K:** Add 600 µl of Proteinase Buffer to dissolve lyophilized Proteinase K. The resulting solution is stable at +4°C for up to 6 months. Storage at –20°C is recommended if the solution will not be used up during this period.

- **Buffer BC4:** Transfer the content of Buffer BC2 to Buffer BC3 and mix well. For dissolving both buffers incubate 5 min at 45ºC. The resulting Buffer BC4 must be stored in the dark and it is stable for 4 months at room temperature. If the Kit will only be used occasionally it is also possible to mix small quantities of Buffer BC3 and Buffer BC2 in a 1:4 ratio (for example, 100 µl Buffer BC3 and 400 µl Buffer BC2). Mix by pipetting up and down.

- **Buffer BC5**: Add 80 ml of ethanol (96-100%) to buffer BC5 concentrate. Store Buffer BC5 at room temperature (20-25°C) for up to one year.

6. HOMOGENIZATION OF SAMPLES

The lysis procedure is most effective when well homogenized, powdered samples are used. To achieve this, we recommend grinding with a pestle and mortar in the presence of liquid nitrogen or vortexing the sample with steel beads*. Commercial homogenizers can also be used. After homogenization and treatment of the sample with lysis Buffer BCF and Proteinase K, mixtures can be cleared easily and effectively by centrifugation.

7. GENERAL REMARKS

- Due to the low DNA content in processed food, this **protocol should be started with up to 200** mg of material.

- The **addition of RNase A** (not included in the kit) may be recommended for RNA-rich samples. Add 10 µl (20 mg/ml stock solution) per 550 µl lysis Buffer BCF in step 2 of the protocol or perform a RNase A digestion in the eluate before further use.

- A **vacuum manifold** can optionally be used for acceleration of washing steps. Loading and elution steps should be done by centrifugation as described in the protocol.

- According to local law regulations different amounts of sample have to be analysed for GMO detection, e.g. up to 1-2 g of sample can be used with upscaled lysis buffer volumes. We recommended to use a single 300 µl aliquot (step 3) of the clear supernatant for further processing with Speedtools Food Columns. Otherwise, prepare 2 aliquots as described in the protocol and load them step by step onto the column.

* ***Homogenization with steel beads (diameter 7 mm)**: Put 4-5 beads and food material together in a 15 ml plastic tube (Falcon), chill the tube in liquid nitrogen and vortex for about 30 sec. Repeat this chilling and vortexing procedure until the entire sample is ground to a powder. Chill the tube once more and remove the beads by rolling them out gently or by using a magnet. Keep the material frozen throughout the whole homogenization procedure. Do not add nitrogen to the tube because it will lead to sticking and loss of sample as it attaches to the beads.*

8. INSTRUCTION FOR USE

A. Standard Protocol

Before starting the protocol prepare Buffer BC4, Buffer BC5, and Proteinase K solution (see section 4). Set two incubators/water baths at 70°C and 65ºC. Preheat lysis Buffer BCF to 65ºC and elution Buffer BCE to 70ºC.

STEP	DESCRIPTION		
1	**HOMOGENIZATION OF SAMPLE** Homogenize about **200 mg material** with a commercial homogenizer.		HOMOGENIZE SAMPLE (200 mg)
2	**CELL LYSIS** Transfer the resulting powder to a 2 ml tube and add **550 µl lysis Buffer BCF (preheated to 65ºC)**. **Mix** carefully 15 sec, add **10 µl Proteinase K** and **mix** again 2-3 sec. *If the lysis buffer volume is not large enough to dissolve the sample completely add more buffer (and Proteinase K proportionally) until sample has been totally resuspended.* Incubate at **65ºC for 30 min**. Afterwards, centrifuge the mixture for **10 min at > 10,000 x *g*** to pellet contaminants and cell debris		+ 550 µl BCF (preheated 65ºC) Mix + 10 µl PROTEINASE K Mix Incubate 65°C, 30 min *10 min, >10,000 x g*
3	**ADJUST DNA BINDING CONDITIONS** Transfer the **clear supernatant** into a new 2 ml centrifuge tube. Add **1 volumen of Buffer BC4** plus **1 volumen ethanol** (96-100%). **Vortex** the mixture for 30 sec.		SUPERNATANT + 1 vol BC4 1 vol ETHANOL Vortex
4	**BIND DNA** For each preparation, take one **column** and its corresponding 2 ml centrifuge tube (provided with the column). **Load the mixture** into the column. Centrifuge **1 min** at **11,000 × *g*. Discard flow-through**. Repeat this step until all the mixture has passed through the column.		Load mixture into a column *1 min, 11,000 × g*
5	**WASH SILICA MEMBRANE** **1st Wash** Place the column in a new 2 ml centrifuge tube (provided). Add **400 µl Buffer BCQW** onto the column. Centrifuge **1 min at 11,000 x *g***. Discard flow-through. **2nd Wash** Place the column in a new 2 ml centrifuge tube (provided). Add **700 µl Buffer BC5** onto the column. Centrifuge **1 min at 11,000 x *g*.** Discard flow-through**.** **3rd Wash** Place the column in a new 2 ml centrifuge tube (provided). Add **200 µl Buffer BC5** onto the column. Centrifuge **2 min at 11,000 x *g*** in order to remove Buffer BC5 completely. Discard flow-through. *Residual ethanol may inhibit enzymatic reactions.*		+ 400 µl BCQW *1 min, 11,000 × g* + 700 µl BC5 *1 min, 11,000 × g* + 200 µl BC5 *2 min, 11,000 × g*
6	**ELUTE DNA** Place the column in a new 1.5 ml centrifuge tube and add **100 µl** elution **Buffer BCE preheated to 70°C.** Dispense buffer directly onto the silica membrane. Incubate at room temperature for 5 min. Centrifuge **1 min at 11,000 x *g*.** The eluate contains your pure DNA sample. ***For alternative elution procedures see section B.***		+ 100 µl BCE (preheated 70ºC) Incubate 5 min RT *1 min, 11,000 × g*

B. Other Elution Procedures

It is possible to adapt elution method and volume of elution buffer to the subsequent application of interest. Use elution Buffer BCE preheated to 70°C for one of the following procedures:

- **High yield** - Perform two elution steps with the volume indicated (2 x 100 µl) in the individual protocol. About 90-100% of bound nucleic acid can be eluted. Finally, combine eluates and measure yield.

- **High concentration** - Perform one elution step with minimal elution volumes (25-50 µl) about 60-80% of bound nucleic acids can be eluted. Concentration of DNA will be higher than with standard elution.

9. TROUBLESHOOTING

Problem	Possible cause and suggestions
No yield or poor DNA yield	***Homogenization of food material was not sufficient*** • For most species we recommended grinding with steel beads or with commercial bead mills, mixers or homogenizers. ***Extraction of DNA from food material during lysis was not sufficient*** • To obtain higher yields of DNA, the incubation time in lysis buffer can be prolonged (up to overnight). ***Sample contains too much RNA*** • Add 10-20 µl RNase A solution to the lysis buffer before heat incubation. If this is not successful, add the enzyme to the cleared lysate and incubate for 30 min at 37ºC. ***Suboptimal elution of DNA from the column*** • The DNA can be either eluted in higher volumes (up to 300 µl) or by repeating the elution step up to three times. Remember that the elution buffer must be preheated to 70ºC prior to elution. • Also check the pH of the used elution buffer, which should be in the range of 8.0-8.5. To ensure correct pH, use supplied elution Buffer BCE.
DNA is degraded	***Sample was contaminated with DNase*** • Check working area and pipettes. ***Centrifugation speed was too high*** • Centrifuge at the speed indicated in the protocol. Higher velocities and prolonged vortexing can lead to shearing of the DNA.
DNA quality is low	***Sample contains DNA-degrading contaminants (e.g. phenolic compounds, metabolites)*** • Repeat washing step with Buffer BCQW.

10. SAFETY INSTRUCTIONS

The following components of the SPEEDTOOLS FOOD DNA EXTRACTION KIT contain hazardous contents. *Wear gloves and goggles and follow the safety instructions given in this section.*

Reagent	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
BC2	Guanidine hydrochloride	Xn*	Harmful if swallowed. Irritating to eyes and skin	R 22-36/38	
BCQW	Guanidine hydrochloride + Ethanol <40%	Xn*	Flammable Harmful if swallowed Irritating to eyes and skin Keep container tightly closed Keep away from sources of ignition	R 10-22-36/38	S 7-16
Proteinase K	Proteinase K, lyophilised	Xn* Xi*	Irritating to eyes,respiratory system and skin May cause sensitisation by inhalation Do not breathe dust Avoid contact with the skin In case of contact with eyes, rinse immediately with plenty of water and seek medical advice Wear suitable protective clothing and gloves	R 36/37/38-42	S 22-24-26-36/37

11. ORDERING INFORMATION

SPEEDTOOLS KIT	50 PREPS	250 PREPS
SPEEDTOOLS **DNA** EXTRACTION KIT	Cat. No. 21.131	Cat. No. 21.132
SPEEDTOOLS **TISSUE DNA** EXTRACTION KIT	Cat. No. 21.136	Cat. No. 21.137
SPEEDTOOLS **RNA VIRUS** EXTRACTION KIT	Cat. No. 21.141	Cat. No. 21.142
SPEEDTOOLS **FOOD DNA** EXTRACTION KIT	Cat. No. 21.176	Cat. No. 21.177
SPEEDTOOLS **PLANT DNA** EXTRACTION KIT	Cat. No. 21.171	Cat. No. 21.172
SPEEDTOOLS **TOTAL RNA** EXTRACTION KIT	Cat. No. 21.211	Cat. No. 21.212
SPEEDTOOLS **PCR CLEAN-UP** KIT	Cat. No. 21.201	Cat. No. 21.202
SPEEDTOOLS **PLASMID DNA** PURIFICATION KIT	Cat. No. 21.221	Cat. No. 21.222

* Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

12. PRODUCT USE RESTRICTION AND WARRANTY

1. Product for research purposes and *in vitro uses* only.
2. No claim or representation is intended for its use to identify any specific microorganism or for clinical use.
3. It is rather the responsibility of the user to verify the use of the kit for a specific application range as the performance characteristic of this kit has not been verified for a specific organism or food type.
4. The kit includes documentation stating specifications and other technical information. Follow the instructions in order to obtain highly pure genomic DNA.
5. BIOTOOLS warrants to meet the stated specifications of the kit. Any product not fulfilling the specifications included in the product sheet will be replaced.
6. BIOTOOLS obligation and purchaser's rights under this warranty are limited to the replacement by BIOTOOLS of any product that is shown defective in fabrication, and that must be returned to BIOTOOLS, freight prepaid, or at BIOTOOLS' option, replacement of the purchasing price.
7. BIOTOOLS does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded). Any complaint on damaged goods during transport must be directed to the handling or transport agent.
8. BIOTOOLS has no responsibility for damages, whether direct or indirect, incidental or consequential of improper or abnormal use of this product. Nor has any responsibility for defects in products or components not manufactured by BIOTOOLS, or against damages resulting from such non-BIOTOOLS components or products.
9. BIOTOOLS makes no other warranty of any kind whatsoever, and specifically disclaims and excludes all other warranties of any kind or nature whatsoever, directly or indirectly, express or implied, including, without limitation, as to the suitability, reproductively, durability, fitness for a particular purpose or use, merchantability, condition, or any other matter with respect to BIOTOOLS products.
10. The warranty provided herein and the data, specifications and descriptions of this kit appearing in BIOTOOLS published catalogues and product literature are BIOTOOLS sole representations concerning the product and warranty. No other statements or representations, written or oral, by BIOTOOLS employees, agent or representatives, except written statements signed by a duly authorized officer of BIOTOOLS are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.
11. Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information. You may also contact your local distributor for general scientific information.
12. Applications mentioned in BIOTOOLS literature are provided for informational purposes only. BIOTOOLS does not warrant that all applications have been tested in BIOTOOLS laboratories using BIOTOOLS products. BIOTOOLS does not warrant the correctness of any of those applications. For more information contact our Technical Dept (info@biotools.eu).

Manufactured by:

BIOTOOLS, Biotechnological & Medical Laboratories, S.A. has been evaluated and certified to accomplish ISO 9001:2000 requirements for the following activities: Research and development of biotechnology products and manufacture of biotechnology and in vitro products.
Valle de Tobalina – 52 – Nave 39, 28021 Madrid – Spain

© 2008 BIOTOOLS, Biotechnological & Medical Laboratories, S.A. All rights reserved.