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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

(Ref. 21.175M/6/7)

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** (plant or animal origin).

Food samples are very heterogeneous and contain different compounds like fat, cocoa, or polysaccharides, which can lead to suboptimal extraction or subsequent processing of DNA. **Speedtools Food DNA Extraction kit** guarantees good recovery for small genomic DNA fragments (<1 kb) out of processed, complex food matrices (e.g., ketchup or spices), which generally have very low DNA contents, as well as poor quality, degraded DNA. Because of this, we recommend the selection of primers, which amplify only short DNA fragments (80-150 bp).

After the food samples have been homogenised, the DNA can be extracted with lysis buffers containing chaotropic salts, denaturing agents, and detergents. The standard isolation ensures lysis using Buffer BCF/Proteinase K. Lysis mixtures should be cleared by centrifugation or filtration in order to remove contaminants and residual cellular debris. The clear supernatant is then mixed with binding buffer and molecular grade ethanol to create conditions for optimal binding to the silica membrane. 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 for subsequent reactions.

2. KIT CONTENTS

REAGENTS	*10 Preps Ref. 21.175M*	*50 Preps Ref. 21.176*	*250 Preps Ref. 21.177*
Buffer BCF	20 ml	100 ml	5 x 100 ml
Buffer BC4	5 ml	30 ml	5 x 30 ml
Buffer BC5 (concentrate)	2 ml	12 ml	5 x 12 ml
Buffer BCQW	5 ml	30 ml	5 x 30 ml
Buffer BCE	2,5 ml	13 ml	5 x 13 ml
Proteinase K (lyophilized)	1.1 mg	6 mg	5 x 6 mg
Proteinase Buffer	300 µl	1.8 ml	5 x 1.8 ml
Speedtools Food Columns (plus Collection Tubes)	10	50	5 x 50
Collection Tubes for the column	1	150	5 x 150
Protocol	1	1	5 x 1

3. KIT SPECIFICATIONS

With the SPEEDTOOLS FOOD DNA EXTRACTION method genomic **DNA is purified from food samples from plant or animal origin.**

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

Table 1. Kit specifications	
Sample material	5-200 mg
Yield	0.1-10 µg
Elution Volume	100 µL
Binding Capacity	30 µg
Time / Prep	30 min/ 6 prep
Fragment Size	>300 bp
Column Type	mini

- **Sample material:** Standard procedure allows processing of up to **200 mg of** material in processed food due to the low DNA content of the samples.
- **Lysis buffer:** The BCF buffer was tested for extraction of DNA from various types of samples including food of plant and animal origin (see Table 2) and appropriate culture medium. Some samples may require to adapt the standard protocol, please contact to our Technical Department (info@biotools.eu).
- **Extraction of bacterial DNA in food samples:** We recommend an overnight pre-culture of sample and appropriate culture medium. Centrifuge an aliquot of the culture and start the preparation with the bacterial pellet.
- **Treatment with RNase A:** RNasa A (not included in the kit) addition may be recommended for RNA-rich samples. Add 10 µL (20 mg/mL stock solution) per 550 µL lysis buffer in step 2 of the protocol or perform an RNasa A digestion in the eluate before further use.
- **Use of vacuum manifold:** 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.
- **GMOs detection:** According to local law regulations different amounts of sample have to be analysed for GMO detection, for example up to **1-2 g of sample** can be used with upscaled lysis buffer (BCF buffer) volume. We recommend using a single 300 µL aliquot of the clear supernatant for further processing with the kit. Otherwise, prepare 2 aliquots as described in the protocol and load them step by step onto the column.

Table 2. 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)

4. HOMOGENISATION AND LYSIS OF SAMPLES

The lysis procedure is most effective when well homogenised, powdered samples are used. This can be achieved with:

- ✓ **Pestle** and mortar in the presence of liquid nitrogen.
- ✓ **Commercial homogenisers**, for example bead mills.
- ✓ **Steel beads** (diameter: 7 mm). Put 4.5 beads and food material together in a 15 mL plastic tube, chill the tube in liquid nitrogen (keep the material frozen throughout the whole homogeneisation procedure) 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 using a magnet. Keep the material frozen throughout the whole homogenisation procedure.

Regarding lysis, the protocol includes slight differences depending on the sample origin:

- ✓ **Fluid samples** such as ketchup, sauce and similar fluid samples (**200 mg** equivalents) can be mixed with lysis **Buffer BCF** (**500-1000 µL**) and incubated with **Proteinase K** as described in the protocol.
- ✓ **Powdered hygroscopic samples**, more lysis Buffer BCF than indicated in the protocol can be used until the lysis solution is at least semi fluid and can be pipetted. Extraction can be improved by **pre-incubation** of sample with lysis Buffer BCF for **1-2 hours**.

5. ELUTION PROCEDURES

It is possible to adapt elution method and volume of elution buffer to the subsequent application of interest:

- **High yield:** Perform two elution steps with **2 x 100 µL** of Elution Buffer BCE and combine eluates. About **90-100%** of bound nucleic acid can be eluted with this protocol.
- **High concentration:** Perform one elution step with minimal volumes of Buffer BCE (**25-50 µL**). About **60-80%** of bound DNA can be eluted, resulting in highly concentrated eluates.

Elution **Buffer BCE** can be replaced by **TE Buffer** or **water**. If water is used, the pH should be checked and adjusted to pH 8-8.5 since deionized water usually exhibits a pH below 7.

6. PREPARATION OF WORKING SOLUTIONS AND STORAGE CONDITIONS

Note: ***Buffers BC4 and BCQW contain guanidine hydrochloride which can form highly reactive compounds when combined with sodium hypochlorite. Do not add sodium hypochlorite or acid solutions directly to the sample-preparation waste.***

- ✓ All kit components can be stored **at room temperature** (18-25°C) through the expiration date printed on the packaging label.
- ✓ If there any precipitate present in the buffers, warm the buffer to 25-37°C to dissolve the precipitate before use.

Before starting any protocol prepare the following reagents:

I. <u>Proteinase K</u>:

- ✓ **10 Preps Format:** Add **110 µl** of **Proteinase Buffer** to dissolve the lyophilized Proteinase K.
- ✓ **50 Preps Format:** Add **600 µl** of **Proteinase Buffer** to dissolve the lyophilized Proteinase K.

*The resulting solution is stable for **6 months** at –20°C.*

II. <u>Buffer BC5:</u>

- ✓ **10 Preps Format:** Add **8 ml of molecular grade ethanol** (96-100%) to the Buffer BC5 concentrate. Mark the label of the bottle to indicate that ethanol was added.
- ✓ **50 Preps Format:** Add **48 ml of molecular grade ethanol** (96-100%) to the Buffer BC5 concentrate. Mark the label of the bottle to indicate that ethanol was added.

*Store the diluted Buffer BC5 at 18-25°C for at least **12 months**.*

7. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

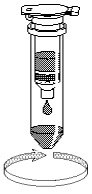
Wear a lab coat, disposable gloves, and protective goggles.

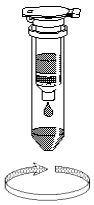
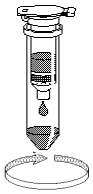
- Appropriate equipment for sample homogenization if required e.g. mortar and pestle, commercial homogenizers, steel beads, etc.
- Microcentrifuge
- Water bath/Incubator
- Vortex
- **Molecular Grade Ethanol 96-100%**
- Microcentrifuge tubes (1.5 ml and 2 ml)
- RNase solution (20 mg/ml)

8. INSTRUCTION FOR USE

Before starting the preparation:

- Check if Buffer BC5 and Proteinase K solution were prepared according to Section 6.
- Set an incubator or water bath to 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	**LYSIS** Transfer the resulting powder to a Collection tube and add **550 µL Buffer BCF** (preheated to 65 ºC). Mix carefully during 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. *<u>Optional</u>: If RNA-free DNA is crucial for downstream applications an RNase digest may be performed: **After** incubation at 65 °C for 30 min, add 10 µL RNase A (20 mg/ml) per 550 µL lysis buffer, mix well, and incubate 30 min at room temperature. Proceed with the protocol with the centrifugation step.*	 	+ 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 centrifuge tube. Add **1 VOL of Buffer BC4** plus **1 VOL molecular grade ethanol** (96-100%). **Vortex** the mixture for 30 sec.		SUPERNATANT + 1 VOL BC4 + 1 VOL MOLECULAR GRADE ETHANOL Vortex
4	**BIND DNA** For each preparation, take one Speedtools Food Column and placed in a Collection tube (provided with the column). **Load 700 µL of mixture** onto the column. Centrifuge **1 min** at **11,000 × *g*. Discard flow-through**. Repeat the procedure of load the remaining sample.		Load mixture into a column *1 min, 11,000 × g*

5	**WASH and DRY SILICA MEMBRANE** **1st Wash** Add **400 µL Buffer BCQW** onto the column. Centrifuge **1 min at 11,000 x *g***. Discard flow-through and place the column back into the Collection tube. **2nd Wash** Add **700 µL Buffer BC5** onto the column. Centrifuge **1 min at 11,000 x *g*.** Discard flow-through and place the column back into the Collection tube **3rd Wash** 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 from Buffer BC5 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 Speedtools Food Column in a new 1.5 ml centrifuge tube and add **100 µL of Buffer BCE (preheated to 70°C).** Dispense buffer directly onto the column. Incubate **5 min** at **room temperature**. Centrifuge **1 min at 11,000 x *g*.** The eluate contains your pure DNA sample. ***For alternative elution procedures see Section 5.***		+ 100 µL BCE (preheated 70ºC) Incubate 5 min RT *1 min, 11,000 × g*

9. TROUBLESHOOTING

Problem	Possible cause and suggestions
DNA yield is low	***Homogenization of food material was not sufficient*** • For most species we recommended grinding with steel beads or with commercial bead mills, mixers or homogenizers. ***Incomplete lysis*** • To improve the yield the lysis could be prolonged up to overnight. ***Sample contains too much RNA*** • Add 10-20 µL RNase A solution to the lysis buffer after 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. 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. ORDERING INFORMATION

SPEEDTOOLS KIT	10 PREPS	50 PREPS	250 PREPS
SPEEDTOOLS **DNA EXTRACTION** KIT	Ref. 21.130M	Ref. 21.131	Ref. 21.132
SPEEDTOOLS **TISSUE DNA EXTRACTION** KIT	Ref. 21.135M	Ref. 21.136	Ref. 21.137
SPEEDTOOLS **RNA VIRUS EXTRACTION** KIT	Ref. 21.140M	Ref. 21.141	Ref. 21.142
SPEEDTOOLS **FOOD DNA EXTRACTION** KIT	Ref. 21.175M	Ref. 21.176	Ref. 21.177
SPEEDTOOLS **PLANT DNA EXTRACTION** KIT	Ref. 21.170M	Ref. 21.171	Ref. 21.172
SPEEDTOOLS **TOTAL RNA EXTRACTION** KIT	Ref. 21.210M	Ref. 21.211	Ref. 21.212
SPEEDTOOLS **PCR CLEAN-UP** KIT	Ref. 21.200M	Ref. 21.201	Ref. 21.202
SPEEDTOOLS **PLASMID DNA PURIFICATION** KIT	Ref. 21.220M	Ref. 21.221	Ref. 21.222

11. 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.
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