DNA Extraction Kit from Food-Stool

BIOPEXT-0609

CONTACT

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BPMR is certified ISO 9001:2015

INTRODUCTION AND PRODUCT DESCRIPTION

The Biopremier DNA Extraction Kit from Food-Stool provides an efficient procedure for the isolation of total DNA from fresh or frozen stool samples and various food samples (raw material and processed food of animal or plant origin) and bacteria. After the food, feed, or stool samples have been homogenized, the DNA can be extracted with the lysis buffer. In case to detect bacterial DNA, a pre-enrichment with the appropriate culture medium is recommended. The bacterial DNA can be extracted with the lysis buffer, after a concentration step by centrifugation.

Lysis occurs by thermal shock and the purification by a series of processes to remove contaminants and residual cellular debris. The clear supernatant is then mixed with the binding buffer, to create conditions for optimal binding to the silica membrane column. The purified DNA is ready-to-use for subsequent reactions like real-time PCR detection and quantification.

Applications:

- **DNA extracted from faecal specimens**
- **DNA from complex matrices, processed food, raw food and animal feed, soya, chocolate, cereals, meat**
- **Detection of specific DNA in animal feed**
- **Detection of genetically modified material in food products (OGM)**
- **Extraction of microbial DNA**
- **DNA Extraction from swabs and sponges**
- **DNA suitable for PCR, real-time PCR, enzymatic reactions**

CONTENTS AND STORAGE

Name Tube	Volume or unit		Storage
	50 preps	250 preps	
Lysis buffer	65 mL	325 mL	RT
Binding buffer	13 mL	65 mL	RT
Proteinase K *	30 mg	2 x 75 mg	- 20ºC
Wash buffer 1 **	16.5 mL	82.5 mL	RT
Wash buffer 2 **	10 mL	50 mL	RT
Elution buffer	10 mL	50 mL	RT
Spin columns	50 units	250 units	RT
Collection Tubes	100 units	500 units	RT

If properly stored, see the expiration date for the stability of the Kit. RT: room temperature.

Notes

* Reconstitute Proteinase K by adding nuclease-free water (Molecular Biology grade) as indicated on vial(s) and stored at -20ºC. It is recommended to do several aliquots to avoid thaw/freeze cycles. At this temperature is stable for 1 year.

** Add ethanol (96-100%) to Wash buffers prior to use as indicated on the bottle(s). Keep the containers closed to avoid the ethanol evaporation.

MATERIAL REQUIRED BUT NOT SUPPLIED

Microcentrifuge tube (1.5 – 2.0 mL; 15 – 50 mL)

Micropipettes and micropipette filter tips (10 – 100 µL and 100 – 1000 µL)

Vortex

Microcentrifuge, able to operate 14.000 rpm.

Heater block (preferably) or Water bath at 37, 65±5 ^{0}C to 95±5 ^{0}C

Powder-free gloves

Ethanol 100 %

Biopremier Real Time Detection kits for meat, fish, GMO, allergens or pathogens (optional)

WARNINGS AND PRECAUTIONS

These products are exclusively for in vitro use.

The test requires qualified staff to prevent the risk of erroneous results.

Do not mix reagents from different batches.

Do not use reagents from other manufacturer's products.

Wear disposable gloves, laboratory coats when handling specimens and reagents.

Use sterile pipette tips with filters.

Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.

Both the Lysis, Bindind buffers and Washing buffer 1 contain guanidine hydrochloride, which can form reactive components when combined with bleach (sodium hypochlorite).

Material Safety Data Sheets (MSDS) are available on request.

Waste must be treated and disposed of in compliance with the appropriate safety standards.

Clean periodically the working space with at least 5% of sodium hypochlorite

It is strongly recommended to have dedicated areas, materials, and equipment for the DNA extraction, preparation of the PCR and post-PCR procedures.

KIT USAGE INFORMATION

The kit contents should be mixed slightly before use.

Under cool environmental conditions, a precipitate may form in the Lysis buffer. In this case, the component should be heated to dissolve precipitate approximately 5 minutes at 37^{0}C and thoroughly shaken prior use.

PROCEDURE

PRELIMINARY PREPARATIONS

- ⚠ Make sure that Proteinase K and Wash Buffers were prepared according to "*Contents and Storage*" section, page 2
- ⚠ Preheat Lysis buffer and Elution buffer to 65-70°C

1. DNA ISOLATION FROM FOOD

(for dairy products see the procedure #2)

Homogenize sample

1. Weigh about **200 mg of food** material and transfer to a microcentrifuge tube.

To obtain a good yield, good homogenization of the sample is necessary. The lysis procedure is most effective when well-homogenized samples are used. We recommend grinding with a pestle and mortar or commercial homogenizers.
In liquid samples use 200 µL directly.
For dairy products see procedure #2 (page 7).

Lyse cells step

2. Pipette **1000 µL of Lysis buffer** (preheated) to the microcentrifuge tube, and add **25 µL of Proteinase K.** Vortex vigorously.

3. Apply a short spin down and incubate for **30 minutes at 65°C.** Vortex one or twice time during incubation (optional).

For samples with hight fat content (ex: chorizo, cheese, ham, etc) an O/N lyse step (16-18h at 65ºC) and the double of Proteinase K (50 ul) may be necessary.

4. Centrifuge at **14.000 rpm for 5 minutes.**

DNA binding step

5. Transfer **500 µL of clear supernatant** to a new microtube with **250 µL of Binding buffer**. Vortex briefly.

On the surface, a layer of fat could appear. To collect a clear supernatant, introduce the pipette tip crossing this surficial layer of fat, only trying to pick up the supernatant liquid with color (and avoid touching with the tip in the pellet).

6. Place the spin column in a 2 mL collection tube.

7. Transfer **600 µL of the mix** to the column, and centrifuge at **12.000 g for 1 minute.** Discard flow-through.

8. Repeat step 7 to **load the remaining sample.**

9. Place the spin column in a new collection tube.

Washing step

10. Add **500 µl of Wash Buffer 1** and centrifuge at **12.000 rpm for 1 minute**. Discard flow-through.

11. Add **700 µl of Wash Buffer 2** and centrifuge at **14.000 rpm for 1 minute**. Discard flow-through.

12. Dry silica membrane. **Centrifuge at 14.000 rpm for 3 minutes.**

Elution step

13. Transfer the spin column to a new 1.5 ml microcentrifuge tube and pipette **100 µL of Elution Buffer** (preheated at 70ºC) onto the membrane. Incubate at room temperature for 2 minutes

Decrease the volume of elution buffer (such as 50 µL) if a higher DNA concentration is desired.

14. Centrifuge at **14.000 rpm for 1 minute**. Discard the spin column and use DNA immediately or store at -20ºC.

2. DNA ISOLATION FROM DAIRY PRODUCTS

⚠ Before starting, preheat Lysis buffer and Elution buffer to 70ºC

Homogenize sample

a. Homogenize the food sample and centrifuge about **50 mL** at **12.000 rpm for 10 minutes**. Discard the supernatant.

To obtain a good yield, good homogenization of the sample is necessary. The lysis procedure is most effective when well-homogenized samples are used. We recommend this procedure for samples for dairy products such as milk, butter and cream cheese or cottage cheese. Other types of cheeses weigh **300 mg** of food material and then and go directly to step 4, of this procedure.

b. Centrifuge more **10 minutes** at **14.000 rpm.** Discard the supernatant.

c. Wash the pellet with 1 mL of nuclease-free water or PBS. Then, centrifuge **3 minutes at 14.000 rpm.**

d. Pipette **1000 µL of Lysis buffer** (preheated) to the microcentrifuge tube, and add **50 µL of Proteinase K.** Vortex vigorously

e. Apply a short spin down and incubate for **30 minutes at 65ºC.** Vortex one ortwice times during incubation

OPTIONAL: O/N lyse step (16-18h at 65ºC)

f. Centrifuge at **14.000 rpm for 5 minutes**

g. Proceed to DNA binding, Washing and Elution steps, in procedure #1 "DNA ISOLATION FROM FOOD" (pages 5 and 6)

3. DNA ISOLATION FROM FRESH OR DRY STOOL

NOTE: It is recommended to begin with big quantities of stool samples when the DNA is not distributed homogeneously or it is in small quantities in the sample.

Homogenize sample

1. Weigh about **200 mg of stool** material and transfer to a microcentrifuge tube. If the sample is liquid transfer 200 µl into the microtube.

For samples of dry stool it is necessary the homogenization of the sample with a manual electric homogenizer.

Lyse cells step

2. Pipette **1000 µL of Lysis buffer** (preheated) to the microcentrifuge tube**.** Vortex vigorously until the stool sample is thoroughly homogenized.

3. Apply a short spin down and incubate for **30-60 minutes at 70^{0}C.** Vortex several times during incubation.

For detection of human DNA it is sufficient to incubate at 70ºC, for detection of bacterial DNA incubate at 80ºC.
For the detection of cells difficult to lyse, as some bacteria or parasites, the incubation temperature you can increase at 95ºC if it was necessary.

4. Centrifuge at **14.000 rpm for 5 minutes.**

5. Transfer **500 µL of clear supernatant** to a new microtube.

DNA binding step

6. Add **25 µl of Proteinase K**. Incubate at 70ºC for 10 minutes.

7. Add **250 µL of Binding buffer**. Vortex briefly.

8. Add the lysate into reservoir of a combined Spin Column–collection tube assembly. **Centrifuge at 10.000 rpm for 60 seconds**. Remove the collection tube.

Washing step

9. Place the Spin column in a clean collection tube, **add 500 µl of Wash Buffer 1**. Centrifuge at **12.000 rpm for 1 minute** and discard the flow-through.

10. Add **700 µl of Wash Buffer 2**. Centrifuge at **14.000 rpm for 1 minute**. Discard the flowthrough.

11. Dry silica membrane. **Centrifuge at 14.000 rpm for 3 minutes**.

Elution step

12. Transfer the spin column to a new 1.5 ml microcentrifuge tube and pipette **100-200 µL of Elution Buffer** (preheated at 70ºC) onto the membrane. Incubate at room temperature for 2 minutes.

    Decrease the volume of elution buffer (such as 50 µL) if a higher DNA concentration is desired.

13. Centrifuge at **14.000 rpm for 1 minute**. Discard the spin column and use DNA immediately or store at -20ºC.

    For good results of PCR use the minimum quantity possible of DNA, the volume should not exceed 10% of the final volume of the mixture of PCR.

4. BACTERIAL DNA ISOLATION FROM ENRICHMENT CULTURE

⚠ Before starting, preheat Lysis buffer and Elution buffer to 70ºC

Concentration step

1. Centrifuge **1 mL** of pre-enrichment or enrichment medium at **14.000 rpm for5 minutes**. Discarded the supernatant

Avoid transferring food debris from the enrichment medium into the microcentrifuge tube.

Lyse cells step

2. Pipette **500 µL of Lysis buffer** (preheated) to the microcentrifuge tube. Vortex vigorously.

3. Apply a short spin down and incubate for **15 minutes at 95ºC**

4. Centrifuge at **14.000 rpm for 5 minutes**

5. Proceed to DNA binding, Washing and Elution steps, in procedure #2 "DNA ISOLATION FROM FOOD" (pages 5 and 6)

5. DNA ISOLATION FROM SWABS AND SPONGES

⚠ Before starting, preheat Lysis buffer and Elution buffer to 70ºC

Lyse cells step

1. Add swab/sponge to **1000 µL of Lysis buffer** (preheated) to the microcentrifuge tube, and add **25 µL of Proteinase K.** Vortex vigorously.

2. Incubate at Room Temperature for 2 hours with shaking.

3. Take out the swab/sponge by pressing it to the wall of the microcentrifuge tube in order to recover all sample.

4. Apply a short spin down and incubate for **30 minutes at 65ºC.** Vortex one or twice time during incubation (optional).

5. Centrifuge at **14.000 rpm for 5 minutes.**

6. Proceed to DNA binding, Washing and Elution steps, in procedure #1 “DNA ISOLATION FROM FOOD” (pages 5 and 6).

WORKFLOW (1. FOOD and 4. ENRICHMENT CULTURE)

	PROCEDURE 1 and 4
1 FOOD HOMOGENIZATION	Homogenize and weight 200 mg of food (for other procedures see the complete protocol)
2 LYSE CELLS	1000 µL Lysis buffer + 25 µL Proteinase K; 65ºC, 30 min (food) **or** 500 µL Lysis buffer 95ºC, 15 min (bacteria) 14.000 rpm, 5 min Take 500 µL of supernatant and continue with step 4
3 BIND DNA	500 µL of supernatant (step 3) 250 µL Binding buffer Load the spin column (max. 600 µL) 10.000 rpm, 1 min
4 WASH	500 µL of Wash buffer 1 12.000 rpm, 1 min 700 µL of Wash buffer 2 14.000 rpm, 1 min 14.000 rpm, 3 min
5 ELUTE DNA	100 µL of Elution buffer (preheated at 70ºC) 14.000 rpm, 1 min

	PROCEDURE 2 (dairy food)
1 FOOD HOMOGENIZATION	Homogenize and weight 50 mL of food (for each type of food see the complete protocol)
2 WASH PELLET	14.000 rpm, 10 min 14.000 rpm, 10 min 1 mL nuclease-free water (or PBS) 14.000 rpm, 3 min
3 LYSE CELLS	1000 µL Lysis buffer + 50 µL Proteinase K 65ºC, 30 min (food) 14.000 rpm, 5 min Take 500 µL of supernatant and continue with step 4
4 BIND DNA	500 µL of supernatant (step 3) 250 µL Binding buffer Load the spin column (max. 600 µL) 14.000 rpm, 1 min
5 WASH	500 µL of Wash buffer 1 12.000 rpm, 1 min 700 µL of Wash buffer 2 14.000 rpm, 1 min 14.000 rpm, 3 min
6 ELUTE DNA	100 µL of Elution buffer (preheated at 70ºC) 14.000 rpm, 1 min

WORKFLOW (3. STOOL)

		PROCEDURE 3
1 FOOD HOMOGENIZATION		Homogenize and weight 200 mg of stool (for other procedures see the complete protocol)
2 LYSE CELLS		1000 µL Lysis buffer 70ºC, 30-60 min **or** 95ºC (bacteria) 80ºC (human DNA) 14.000 rpm, 5 min Take 500 µL of supernatant
3 BIND DNA		500 µL of supernatant + 25 µL Proteinase K (Incubate at 70ºC for 10 minutes) +250 µL Binding buffer Load the spin column 10.000 rpm, 1 min
4 WASH		500 µL of Wash buffer 1 12.000 rpm, 1 min 700 µL of Wash buffer 2 14.000 rpm, 1 min 14.000 rpm, 3 min
5 ELUTE DNA		100 µL of Elution buffer (preheated at 70ºC) 14.000 rpm, 1 min

TROUBLESHOOTING

Trouble	Possible Reason	Solution Suggest
Low DNA yield or low DNA purity	Inappropriate storage conditions	The Kit should be stored between +15 and +25°C, except Proteinase K should be stored at -20°C. The tube and bottle caps must be tightly sealed after each use to maintain the pH values and stability of the kit components, and to prevent contamination
	Chemicals and sample are not mixed well	The sample should be thoroughly mixed after each chemical addition
	Poor elution	Incubating the column with elution buffer for 2 minutes at 70 ºC may increase the yield Elute the DNA with 50 µL of elution buffer
No amplification after PCR/qPCR run or the enzymatic reactions are not working	Alcohol residue in DNA isolate	The remaining ethanol after washing steps should be removed by centrifuging the column at 14.000 rpm for 3 minutes

QUALITY CONTROL

Each lot of the kit is tested against predetermined specifications to ensure consistent product quality.

TRADEMARK, DISCLAIMER AND PRODUCT USE RESTRICTION

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law. BPMR kit handbooks and user manuals can be requested from BPMR or your local distributor.

The use of this product signifies the agreement of any purchaser or user to the following terms: The kit must be used solely in accordance with the respective Instructions for Use. BPMR grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this kit except as described in the Instructions for Use. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

The kit components are intended exclusively for *in vitro* use, and for research purposes only! BPMR products are intended for general laboratory use only! Molecular Biology procedures, such as DNA extractions and PCR amplification, require qualified staff to prevent the risk of erroneous results. It is strongly recommended to have dedicated areas, materials and equipment for the DNA extraction, preparation of the PCR and post-PCR procedures. The workflow in the laboratory should proceed in a uni-directional manner, from the Extraction Area to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed the previous step. The user should always read all the instructions provided with the product before running the assay. Not mix reagents from different batches. Not use reagents from other manufacturer’s products. Wear disposable gloves, laboratory coats when handling specimens and reagents. Use sterile pipette tips with filters. Waste must be treated and disposed of in compliance with the appropriate safety standards.

ADDITIONAL INFORMATION

For additional information, technical support or troubleshooting please contact: tech.support@biopremier.com

ORDERING INFORMATION

Biopremier offers a large selection of products. Visit www.biopremier.com or contact sales.support@biopremier.com for more detailed product information.

Reference	Product	Quantity
BIOPFS-0001	REAL TIME DETECTION KIT *Salmonella* spp.	100 rxn
BIOPFS-0003	REAL TIME DETECTION KIT *Listeria monocytogenes*	100 rxn
BIOPFS-0004	REAL TIME DETECTION KIT *Vibrio* spp.	100 rxn
BIOPFS-0005	REAL TIME DETECTION KIT *Campylobacter jejuni*	100 rxn
BIOPFS-0047	REAL TIME DETECTION KIT *Cronobacter* spp.	100 rxn
BIOPSFS-0002	SUPREME REAL TIME DETECTION KIT *E. coli*	50 + 50 rxn
BIOPSFS-0059	SUPREME REAL TIME DETECTION KIT *E. coli* O157:H7 / O157	100 rxn
BIOPFS-0049	REAL TIME DETECTION KIT Celery	100 rxn
BIOPFS-0050	REAL TIME DETECTION KIT Sesame	100 rxn
BIOPFS-0051	REAL TIME DETECTION KIT Peanuts	100 rxn
BIOPFS-0052	REAL TIME DETECTION KIT Soy bean	100 rxn
BIOPFS-0053	REAL TIME DETECTION KIT Hazelnuts	100 rxn
BIOPFS-0054	REAL TIME DETECTION KIT Cashew	100 rxn
BIOPFS-0055	REAL TIME DETECTION KIT Lupin	100 rxn
BIOPFS-0060	REAL TIME DETECTION KIT Mustard	100 rxn
BIOPFS-0016	REAL TIME DETECTION KIT P-35S and T-NOS	100 + 100 rxn
BIOPFS-0017	REAL TIME DETECTION KIT P-35S, T-NOS and P-FMV	50 + 50 + 50 rxn
BIOPFS-0018	REAL TIME DETECTION KIT P-FMV	100 rxn
BIOPSFS-0006	SUPREME REAL TIME DETECTION KIT Cow	100 rxn
BIOPSFS-0007	SUPREME REAL TIME DETECTION KIT Swine	100 rxn
BIOPSFS-0008	SUPREME REAL TIME DETECTION KIT Horse	100 rxn
BIOPSFS-0009	SUPREME REAL TIME DETECTION KIT Duck	100 rxn
BIOPSFS-0010	SUPREME REAL TIME DETECTION KIT Chicken	100 rxn
BIOPSFS-0011	SUPREME REAL TIME DETECTION KIT Turkey	100 rxn
BIOPSFS-0012	SUPREME REAL TIME DETECTION KIT Goat	100 rxn
BIOPSFS-0013	SUPREME REAL TIME DETECTION KIT Sheep	100 rxn
BIOPSPS-056	SUPREME REAL TIME DETECTION KIT Atlantic Cod	100 rxn
BIOPSPS-057	SUPREME REAL TIME DETECTION KIT Pacific Cod	100 rxn
BIOPSPS-058	SUPREME REAL TIME DETECTION KIT Alaska pollack	100 rxn
BIOPEXT-0609	DNA Extraction Kit from food	50 / 250 preps
BIOPEXT-0400	DNA Rapid Extraction buffer	100 / 250 preps