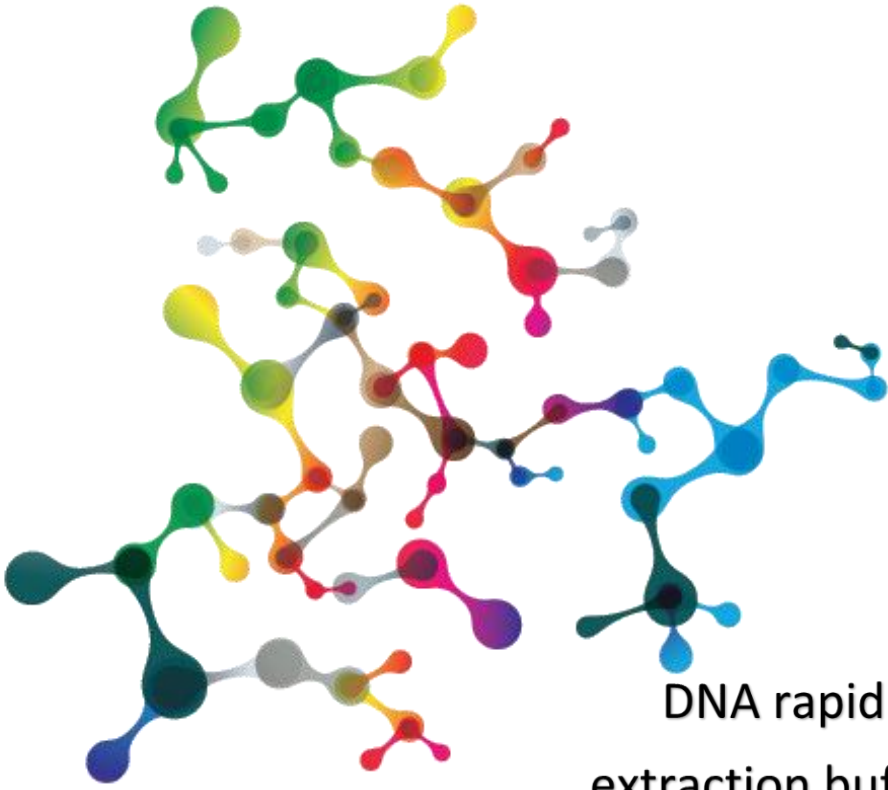
BIOPREMIER



DNA rapid extraction buffer

BIOPEXT-0400

CONTACT

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BPMR is certified ISO 9001:2015

INTRODUCTION AND PRODUCT DESCRIPTION

The BIOPREMIER Rapid DNA extraction buffer provides a simple way to prepare and extract samples for bacterial and yeast DNA testing. For bacteria microorganisms, a pre-enrichment with the appropriate culture medium is recommended and the bacterial DNA can be extracted with the lysis buffer, after a concentration step by centrifugation from samples in food, water, feed etc. in accordance with the standard methods (ISO 6579, ISO 11290, ISO 16654 and so on). For yeast microorganisms an additional step with the enzyme lyticase is recommended.

Lysis occurs by thermal shock and a centrifugation step removes part of residual cellular debris. The supernatant could be used directly for subsequent reactions like real-time PCR detection or after a dilution step to remove residual PCR inhibitors.

CONTENTS AND STORAGE

Name Tube	Volume		Storage
	100 - 200 preps	250-500 preps	
Lysis buffer	20 mL	50 mL	RT

Store at room temperature (RT)

MATERIAL REQUIRED BUT NOT SUPPLIED

Microcentrifuge tube (1.5 – 2.0 mL)
Micropipettes and micropipette filter tips (10 – 100 µL and 100 – 1000 µL)
Vortex
Microcentrifuge, able to operate up to 14.000 g.
Heater block (preferably) or Water bath at 95±5 ^{o}C
Powder-free gloves

REAGENTS REQUIRED BUT NOT SUPPLIED

BIOPREMIER Real Time Detection kits for pathogens (optional)

For Beverages (optional):
Lyticase
Phosphate Buffered Saline (PBS)

WARNINGS AND PRECAUTIONS

These products are exclusively for in vitro use and for food use only
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.
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, the precipitate may form in some kit components. In this case, the component should be heated to dissolve precipitate approximately 5 minutes at 37^{o}C and thoroughly shaken prior use.

1. BACTERIAL DNA ISOLATION FROM PRE- OR ENRICHMENT CULTURE

Concentration step

1. Centrifuge **1 mL** of pre-enrichment or enrichment medium at **>10.000 g for 5 minutes**. Discarded the supernatant.

Avoid transferring food debris from the enrichment medium into the microcentrifuge tube.
OPTIONAL: Performed a washing step before the step 2: Add 1mL of sterile PBS, resuspended the pellet and centrifuge at > 10.000 g for 3 minutes. Discarded the supernatant. Continues to the step 2.

Lyse cells step

2. Pipette **100- 200 µL of Lysis buffer** to the microcentrifuge tube and resuspended the pellet. Vortex vigorously.

3. Apply a short spin down and incubate for **10-15 minutes at 95-100°C**.

4. Centrifuge **>10.000 g for 5 minutes**.

5. Transfer the **clear supernatant** to a new microcentrifuge tube.

6. Use DNA immediately or store at -20°C if you want to reuse it. Homogenize and centrifuge >10.000 g for 2 minutes before reusing.

2. BACTERIAL DNA ISOLATION FROM ISOLATED COLONIES

1. Pipette **100 µL of Lysis buffer** to the microcentrifuge tube.
2. Select a small loopful of cells on a culture plate and **suspend the cells** in the lysis buffer reagent. Vortex vigorously.
3. Apply a short spin down and incubate for **10-15 minutes at 95-100ºC**.
4. Centrifuge at **>10.000 g for 5 minutes**.
5. Transfer the **clear supernatant** to a new microcentrifuge tube.
6. Use DNA immediately or store at -20ºC if you want to reuse it. Homogenize and centrifuge >10.000 g for 2 minutes before reusing.

3. DNA ISOLATION FROM BEVERAGES

Concentration step

1. Centrifuge up to **50 mL** at **maximum speed g for 10 minutes**. Decant the liquid carefully, leave about 1 mL in the cone of the tube. (Centrifuge volumes between 1 mL and 50 mL)

Wash step

2. Mix liquid in the cone with a pipette tip and transfer it completely into a 1.5mL microtube and centrifuge for 3 minutes at **>10.000 g**.
3. With a pipette tip remove **all the supernatant**.
4. Pipette **700 µL of PBS sterile** and resuspend the pellet by vortexing or pipetting up and down. Centrifuge for 3 minutes at **>10.000 g.**

5. With a pipette tip remove **all the supernatant. Repeat the step 4**, one more time (wash buffer and centrifugation).

6. With a pipette tip remove **all the supernatant**.

Lyse cells step

7. Pipette **100 µL of Lysis buffer** to the microcentrifuge tube and resuspended the pellet by vortexing or pipetting up and down. (If the pellet is not completely covered, increase the volume of lysis buffer (no more than 200 µL)

8. Add **3 µL of Lyticase** (15U)**.** (Add 3ul of lyticase per each 100 µL of lysis buffer added in step 7).

9. Vortex and incubate for **15 minutes at 37°C**.

10. Incubate for **10 - 15 minutes at 95 - 100°C**.

11. Centrifuge **>10.000 g for 5 minutes.**

12. Transfer the **clear supernatant** to a new microcentrifuge tube.

13. Use DNA immediately or store at -20°C if you want to reuse it. Homogenize and centrifuge >10.000 g for 2 minutes before reusing.

14. For the BIOPREMIER kits, BIOPFS-0019 or BIOPFS-0020, we recommend using **2 µL of the DNA extract** or a 10-fold dilution with PCR-grade water (e.g. 5 µL of the DNA extract + 45 µL ddH_2O) and then use 2 µL of the diluted DNA extract. (This 10-fold dilution ensures the dilution of qPCR inhibitors. If the DNA concentration is too low, one can dilute less (e.g. 5-fold or undiluted).

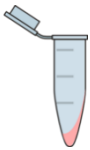
WORKFLOW (1. ENRICHMENT CULTURE)

		PROCEDURE 1
1 CELL CONCENTRATION		Collect 1 mL of medium >10.000 x g, 5 min
2 LYSE CELLS		100- 200 µL Lysis buffer 95/100ºC, 10-15 min >10.000 x g, 5 min Take of supernatant

WORKFLOW (2. ISOLATED COLONIES)

		PROCEDURE 2
1 LYSE CELLS		Select a small loopful of cells 100 µL Lysis buffer 95/100ºC, 10-15 min >10.000 x g, 5 min Take of supernatant

WORKFLOW (3. DNA FROM BEVERAGES)

		PROCEDURE 3
1 CELL CONCENTRATION		Collect 1 - 50 mL Max speed, 10 min Decant the supernatant
2 WASH CELLS		>10.000 x g, 3 min Remove the supernatant 700 µL PBS >10.000 x g, 3 min Remove the supernatant **Repeat**: 700 µL PBS >10.000 x g, 3 min Remove the supernatant
3 LYSE CELLS		100 - 200 µL Lysis buffer 3 - 6 µL Lyticase 37ºC, 15 min 95-100ºC, 10 - 15 min >10.000 x g, 5 min Use DNA directly or dilluted

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. The bottle cap 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
DNA absorbance value (A260) is too high	If the DNA concentration is too high or the impurities in the DNA obtained	The DNA obtained should be diluted with molecular grade water (such as 2 or 10 fold etc.) prior to PCR
No amplification after PCR/qPCR run	The presence of reaction inhibiting impurities in the obtained DNA	When transferring DNA after centrifugation, care should be taken to remove the DNA from the top of the liquid The DNA obtained should be diluted with molecular grade water (such as 2 or 10 fold etc.) prior to PCR

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-0019	REAL TIME DETECTION KIT Brettanomyces/Dekkera	100 rxn
BIOPFS-0020	REAL TIME DETECTION KIT Zygosaccharomyces bailii	100 rxn