ExtractNow™ Food Control

Extraction of bacterial DNA from stomacher bag cultures

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Order No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

The *ExtractNow™ Food Control* kit is a spin column based DNA extraction method for nucleic acid purification from a broad range of different enrichment broths as starting material. Using a cutting -edge chemistry, the duration of the DNA purification is reduced to a minimum.

PRINCIPLE OF THE METHOD

The method is simple and consists of four general steps: (1) sample lysis, (2) binding of DNA on *Spin Columns*, (3) removal of residual contaminants and inhibitors, and (4) elution of purified DNA. The procedure does not require phenol/chloroform extraction and needs minimal handling time. The kit's chemistry facilitates fast purification of genomic DNA in approximately one hour (including lysis). Yield and quality depend on type of bacteria and density of culture.

CONTENT

Each kit contains reagents for 10 or 50 extractions. The expiry date of the unopened package is marked on the package label. Store the lyophilized *Proteinase K* at 4 °C and all other components at room temperature (15 to 30 °C). Before every use, ensure that all components have room temperature. Dissolve any precipitates in the solutions by moderate warming.

Kit component	10 extractions (609-1010)	50 extractions (609-1050)
Spin columns (blue)	10 units	50 units
Collection tubes	40 units	4 x 50 units
Lysis Tubes	10 units	50 units
Resuspension A	12 ml	60 ml
Resuspension B	2 ml	8 ml
Lysis Buffer F	2 x 2 ml	15 ml
Binding Buffer D	2 x 2 ml	20 ml
Wash Buffer C	3 ml (add 3 ml ethanol (>96%) before first use)	15 ml (add 15 ml ethanol (>96%) before first use)
Wash Buffer F	3 ml (add 7 ml ethanol (>96%) before first use)	15 ml (add 35 ml ethanol (>96%) before first use)
Elution Buffer A	2 ml	10 ml
Proteinase K	1 x 6 mg (add 0.3 ml of ddH_2O)	1 x 30 mg (add 1.5 ml of ddH_2O)

The Lot-specific QC certificate (*Certificate of Analysis*) can be downloaded from our website (www.minerva-biolabs.com).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The *ExtractNow™ Food Control* kit contains reagents for isolating DNA from enrichment broths. Additional consumables and equipment is supplied by the user:

- Ethanol > 96 % abs.
- 1.5 ml and 2 ml tubes
- Microcentrifuge
- Heat block or thermomixer for 1.5 ml reaction tubes
- Pipettes with corresponding filter tips (100 and 1000 µl)
- Bidest water
- RNAse A, 100 mg/ml (optional)

SPECIMEN

Best results are obtained with fresh or fresh frozen material. Repeated freeze/thaw-cycles must be avoided as it is detrimental to DNA integrity.

In order to obtain best results it is also important not to use to overload the spin collumns. The maximum amounts of starting material is a bacterial cell pellet from 1 ml enrichment broth from a standard stomacher bag.

PRECAUTIONS

The *ExtractNow™ Food Control* kit is for research use only. The kit should be used by trained laboratory staff only.

All samples should be considered as potentially infectious and handled with all due care and attention. Always wear suitable lab coat, disposable gloves, and protective goggles.

Do not add bleach or acidic solution to the sample preparation waste. See safety data sheets for detailed information.

In case of contact, flush eyes or skin with water. Do not swallow components of the kit. Clean with suitable laboratory detergent and water, if any liquid is spilt.

This kit can be disposed of as municipal waste according to local guidelines.

IMPORTANT NOTES

- Dissolve the *Proteinase K* with the given volume of ddH_2O and mix thoroughly by pipetting. Dissolved *Proteinase K* must be stored at ≤ –18°C. Repeated freeze/thaw cycles will reduce the enzyme activity. We therefore recommend to prepare aliquots.
- Set up the heat block between 95 and 99 °C and for the following steps to 50 °C.
- Ensure that ethanol was added to *Wash Buffer C and Wash Buffer F.* Do not use other alcohol apart from ethanol as it will lead to inconsistent yields.
- The centrifugation steps should be carried out at room temperature.
- The reagents supplied should not be mixed with reagents from different Lot but used as an integral unit. The reagents of the kit must not be used beyond shelf life.
- Follow the exact protocol. Any deviation may affect the results.

PROCEDURE – OVERVIEW

+ up to 1 ml of culture broth

10,000 × g for 2 min

remove supernatant

+ 1 ml Resuspension A

or pipet up and down

10,000 × g for 2 min

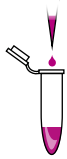
remove supernatant

+ 100 µl Resuspension B

or pipet up and down

transfer sample into a Lysis Tube

+ 95-99 °C for 20 min



+ 200 ml Lysis Buffer F

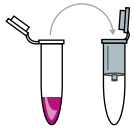
+ 25 µl Proteinase K

mix vigorously

+ 50 °C for 30 min

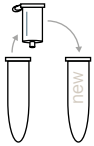
+ 300 µl Binding Buffer D

mix vigorously



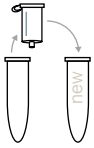
transfer to spin column

10,000 × g for 2 min



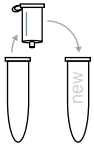
+ 500 µl Wash Buffer C

10,000 × g for 1 min

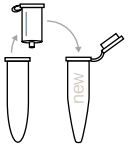


+ 650 µl Wash Buffer F

10,000 × g for 1 min



at max speed for 2 min



+ 100 µl Elution Buffer A

for 1 min

6,000 × g for 1 min

DNA ready for PCR

+ add vortex incubate shake centrifuge

PROCEDURE - STEP BY STEP

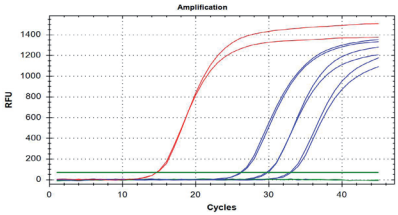
⇒ Before first use reconstitute *Wash Buffer C* and *Wash Buffer F* with absolute ethanol
⇒ Rehydrate the *Proteinase K* with water.
⇒ Set the heat block between 95 °C to 99 °C.

1.	After standard cultivation in a stomacher bag, transfer up to 1 ml of the culture broth into a 2.0 ml reaction tube. Centrifuge at 10,000 x g for 2 min to pellet the bacterial cells. Remove the supernatant completely.
2.	Add 1 ml *Resuspension A* to the pellet. Re-suspend the cell pellet by pipetting up and down or by vortexing. Centrifuge at 10,000 x g for 2 min to pellet the bacterial cells again. Remove the supernatant completely.
3.	Add 100 µl *Resuspension B* to the pellet. Re-suspend the cell pellet by pipetting up and down or by vortexing. After resuspension transfer the sample into a Lysis Tube.
4.	Incubate the *Lysis Tube* in a thermal mixer permanently shaking at 95 °C to 99 °C for 20 min. After incubation let the sample equilibrate at room temperature.
5.	Add 200 ml *Lysis Buffer F* and 25 µl *Proteinase K*. Optional: Add 3 µl RNase A (from stock solution 100 mg/ml; not included in the kit) to remove the RNA. Mix vigorously by pulsed vortexing for 5 sec and incubate at 50 °C for 30 min. We recommend the use of a thermomixer for a permanent shaking of the samples as it will increase the DNA yield. Alternatively, vortex the samples 3 to 4 times during the incubation.
6.	Add 300 µl *Binding Buffer D* to the *Lysis Tube* and mix thoroughly by pipetting up and down to get a homogeneous solution.
7.	Transfer the sample to a spin column placed in a collection tube. Centrifuge at 10,000 x g for 2 min. Note: If the solution has not completely passed through the spin column, centrifuge again at a higher speed or prolong the centrifugation. Discard the *Collection Tube* with the flow-through and place the *Spin Column* into a new *Collection Tube.*
8.	Add 500 µl *Wash Buffer C* and centrifuge at 10,000 x g for 1 min. Discard the *Collection Tube* with the flow-through and place the *Spin Column* into a new *Collection Tube*.

9.	Add 650 µl *Wash Buffer F* and centrifuge at 10,000 x g for 1 min. Discard the *Collection Tube* with the flow-through and place the *Spin Column* into a new *Collection Tube*.
10.	Centrifuge at max. speed for 2 min to remove all traces of *Wash Buffer F*. Discard the collection tube and place the spin column in a new 1.5 ml tube.
11.	Add 100 µl *Elution Buffer A* and incubate at room temperature for 1 min. Note: The DNA may be eluted 2-times with 50 µl of Elution Buffer A. This should increase the yield of DNA.
12.	Centrifuge at 6000 x g for 1 min. Note: The DNA can be eluted with a lower or a higher volume of *Elution Buffer A* (depends on the expected yield of genomic DNA). Elution with lower volumes of *Elution Buffer A* will increase the final concentration of DNA. Store the extracted DNA at 4 °C or below –18 °C for long time storage.

Food Control™ qPCR

Detect foodborne pathogens with easy interpretable lateral flow evaluation.



Features

Target	• Salmonella enterica – invasion protein (invA) gene • Yersinia enterocolitica – heat-stable enterotoxin A gene • Shigella spp. – invasion plasmid antigen (ipaH6) gene • Campylobacter spp. – acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase (lpxA) gene • Clostridium perfringens – phospholipase C alpha toxin (plc) gene • Shiga Toxin 1 – stx1 gene • Shiga Toxin 2 – stx2 gene • Escherichia coli O157 – wbdR gene • Escherichia coli O104 – wckD gene • Listeria spp. – invasion associated protein p60 (iap) gene • Listeria monocytogenes – listeriolysin O (hly) gene • Salmonella spp. – spacer-region between 16S and 23S RNA genes
Sensitivity	Down to 10 DNA copies/assay.
Principle	TaqMan® assay based on FAM and HEX labeled probes.
Content	qPCR Mix Species Mix Rehydration Buffer PCR Grade Water Internal Control Positive Control
Sample Requirements	Isolated total DNA from potentially contaminated food serves here as starting material, typically after pre-cultivation of the sample growth medium.
Intended Use **Time to Result**	For research use only! 150 minutes
Cycler	• qTOWER (Analytik Jena) • TOptical (Analytik Jena) • Rotor-Gene® (Qiagen) • Rotor-Gene®6000 (Qiagen) • LightCycler® (Roche Diagnostics) • Mastercycler® ep reaplex (Eppendorf) • CFX Connect™ (Bio-Rad) • StepOnePlus™, ABI 7500 (Applied Biosystem®) • Mx3005P (Agilent Technologies)

Related Products

qPCR Kits for Food Control		
11-03-XX-025	FoodControl™ LFA	25 tests
11-04-XX-025	FoodControl™ LFA+	25 tests
qPCR Kits for Meat Identification		
12-01-005/-020/-040	Meat ID™ Screen	5/20/40 tests
12-02-025/-100	Meat ID™ Halal	25/100 tests
qPCR Kits for Vegan Control		
12-05-025/-100	VeganControl™ OneStep	25/100 tests
12-06-024/-096/-240	VeganControl™ Advance	24/96/240 tests
DNA Extraction kits		
56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions
56-2096	Venor®GeM Sample Preparation Kit - IP C16	96 extractions
601-1010/1050/1200	ExtractNow™ DNA Mini Kit	10/50/200 extractions
602-1010/1050/1200	ExtractNow™ Blood DNA Mini kit	10/50/200 extractions
603-1010/1050/1200	ExtractNow™ RNA Mini kit	10/50/200 extractions
604-1010/1050/1200	ExtractNow™ Cleanup kit	10/50/200 extractions
605-1010/1050/1200	ExtractNow™ Plasmid Mini kit	10/50/200 extractions
606-1010/1050/1200	ExtractNow™ Virus DNA/RNA kit	10/50/200 extractions
607-1010/1050	ExtractNow™ Vegan Control	10/50 extractions
608-1010/1050	ExtractNow™ Meat ID	10/50 extractions
609-1010/1050	ExtractNow™ Food Control	
Mycoplasma Contamination Control PCR kits		
11-1025/1050/1100/1250	Venor®GeM Classic Mycoplasma Detection Kit	25/50/100/250 tests
11-7024/7048/7096/7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 tests
11-8025/8050/8100/8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/50/100/250 tests
12-1025/1050/1100/1250	Onar® Bacteria Detection Kit	25/50/100/250 tests
11-9025/9100/9250	Venor®GeM qEP Mycoplasma Detection Kit	25/100/250 tests
PCR Clean™ (formerly DNA Remover™)		
15-2025/2200	DNA Decontamination Reagent, spray bottle/refill bottles	250 ml/4x 500 ml
15-2201	Wipes	120 wipes in a dispenser box
15-2202	Wipes, refill packs	5 x 120 wipes in a bag
Lab Clean		
15-4100	Molecular Microbiology Lab Cleaner	1 litre
Mycoplasma Off™		
15-1000	Surface Disinfectant Spray, spray bottle	1000 ml
15-5000	Surface Disinfectant Spray, refill bottles	5 x 1000 ml
15-1001	Surface Disinfectant Wipes in dispenser box	120 wipes
15-5001	Surface Disinfectant Wipes, refill pack	5 x 120 wipes
ZellShield™		
13-0050/0150	Contamination Prevention Reagent 100x concentrate	50 ml/ 3 x 50 ml
WaterShield™		
15-3015/3020/3050	Water Disinfection Additive for incubators and water baths, 200x concentrate	30 x 5 ml/3 x 50 ml/500 ml

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