

# ExtractNow™ Food Control

Extraction of bacterial DNA from stomacher bag cultures

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## INSTRUCTIONS FOR USE

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**FOR USE IN RESEARCH AND QUALITY CONTROL**

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## Symbols

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**Lot No.**



**Cat. No.**



**Expiry date**



**Storage temperature**



**Number of reactions**



**Manufacturer**

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## 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. Using a cutting-edge chemistry, the duration of the DNA purification is reduced to a minimum.

## TEST PRINCIPLE

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 the 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 +2 - +8 °C and all other components at room temperature (+15 to +30 °C). Dissolve any precipitates in the solutions by moderate warming.

Component	Quantity	
	10 extractions Cat. No. 609-1010	50 extractions Cat. No. 609-1050
Spin Columns (blue)	10 units	50 units
Collection Tubes	4 × 10 units	4 × 50 units
Lysis Tubes	10 units	50 units
Resuspension A	12 ml	60 ml
Resuspension B	2 ml	8 ml
Lysis Buffer F	2 × 2 ml	15 ml
Binding Buffer D	2 × 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 × 6 mg (add 0.3 ml of ddH <sub>2</sub> O)	1 × 30 mg (add 1.5 ml of ddH <sub>2</sub> O)

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website ([www.minerva-biolabs.com](http://www.minerva-biolabs.com) / [www.minervabiolabs.us](http://www.minervabiolabs.us)).

## **USER-SUPPLIED CONSUMABLES AND EQUIPMENT**

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

- Ethanol > 96 % abs. (molecular biology grade)
- 1.5 ml and 2 ml tubes
- Microcentrifuge, vortex
- Heat block (possibly with shaking function) or thermomixer for 1.5 ml and 2 ml reaction tubes
- Pipettes with corresponding filter tips (100 and 1000  $\mu$ l)
- ddH<sub>2</sub>O to dissolve the Proteinase K
- RNase A, 100 mg/ml (optional)

## **SPECIMEN**

Best results are obtained with fresh or freshly frozen material. Repeated freeze-thaw cycles of the starting material must be avoided, as this is detrimental to DNA integrity.

For a successful DNA extraction, it is also essential not to overload the spin columns. The maximum amount of starting material corresponds to a bacterial cell pellet obtained 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<sub>2</sub>O and mix thoroughly by pipetting.

The 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.

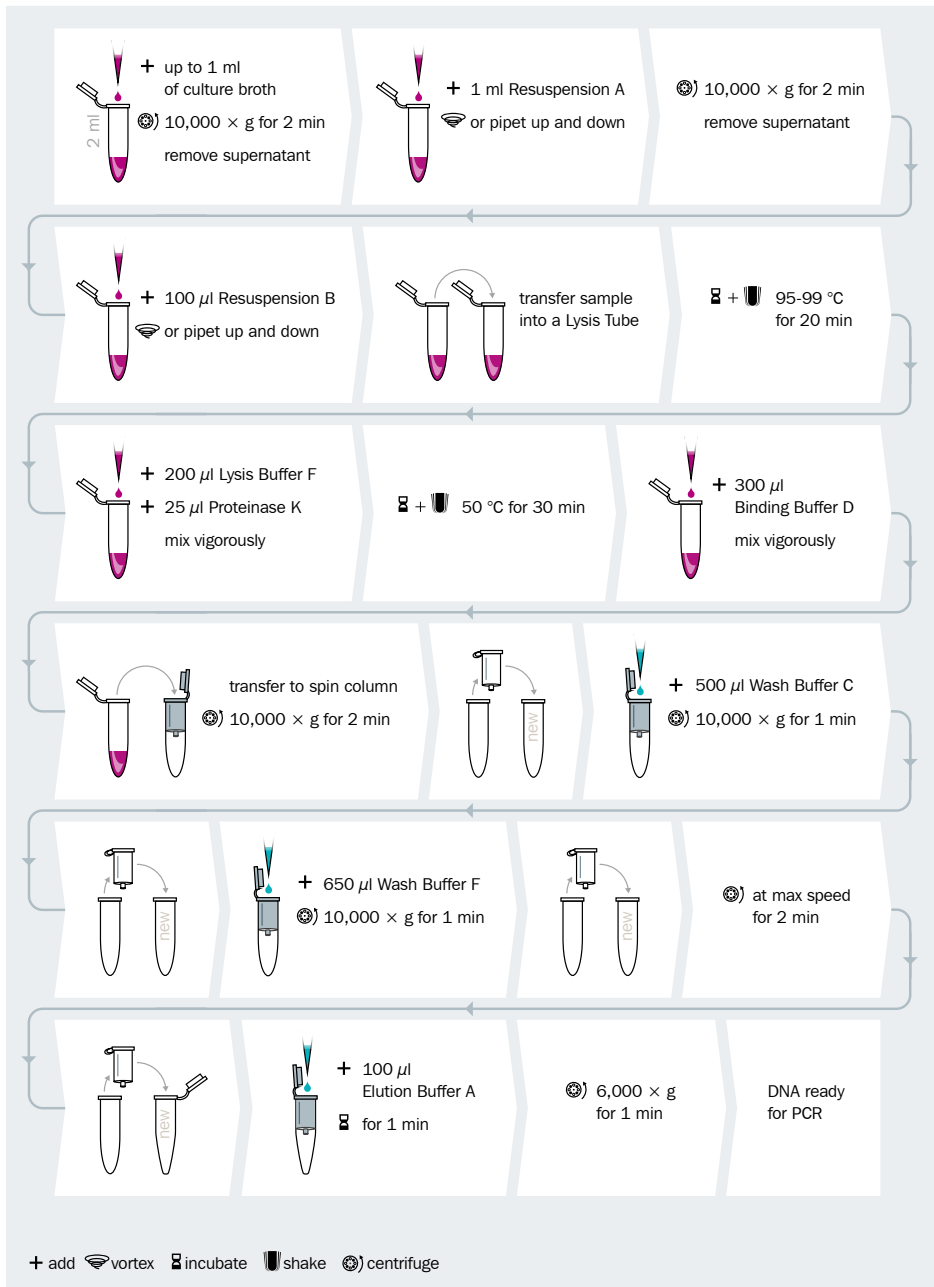
⇒ Ensure that ethanol was added to Wash Buffer C and Wash Buffer F. Do not use any alcohol other than ethanol as it will lead to inconsistent yields.

⇒ The centrifugation steps should be carried out at room temperature.

⇒ The reagents supplied should not be mixed with reagents from different lots 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



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## PROCEDURE - STEP BY STEP

- ⇒ Before first use, reconstitute Wash Buffer C and Wash Buffer F with absolute ethanol
- ⇒ Rehydrate the Proteinase K with water as indicated in the „Content“ table. Please read also the chapter „Important Notes“.
- ⇒ Set the temperature of the heat block between 95 °C and 99 °C, then decrease to 50 °C for step 5.

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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  $10,000 \times g$  for 2 min to pellet the bacterial cells. Discard the supernatant, entirely.

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  2. Add 1 ml Resuspension A to the pellet and resuspend by pipetting up and down or by vortexing. Centrifuge at  $10,000 \times g$  for 2 min to pellet the bacterial cells again. Discard the supernatant, entirely.

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  3. Add 100  $\mu$ l Resuspension B to the pellet and resuspend by pipetting up and down or by vortexing. Once the sample is dissolved, transfer it to a Lysis Tube.

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  4. Incubate the Lysis Tube in a thermomixer with continuous shaking at 95 °C to 99 °C for 20 min. After incubation, equilibrate the sample at room temperature.

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  5. Add 200  $\mu$ l Lysis Buffer F and 25  $\mu$ l Proteinase K. Optional: Add 3  $\mu$ l RNase A (stock solution 100 mg/ml; not provided) to remove RNA. Mix vigorously by pulsed vortexing for 5 sec and incubate at 50 °C for 30 min. We recommend continuous shaking during this step to increase the DNA yield. Alternatively, vortex the samples 3 to 4 times during the incubation.

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  6. Add 300  $\mu$ l Binding Buffer D to the Lysis Tube and mix thoroughly by pipetting up and down in order to obtain a homogeneous solution.

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  7. Transfer the sample to a Spin Column, placed on a Collection Tube. Centrifuge at  $10,000 \times g$  for 2 min. Discard the Collection Tube with the flow-through and place the Spin Column on a new Collection Tube. Note: If the column still contains some liquid, it may be necessary to prolong the centrifugation or centrifuge at a higher speed.

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  8. Add 500  $\mu$ l Wash Buffer C and centrifuge at  $10,000 \times g$  for 1 min. Discard the Collection Tube with the flow-through and place the Spin Column on a new Collection Tube.

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  9. Add 650  $\mu$ l Wash Buffer F and centrifuge at  $10,000 \times g$  for 1 min. Discard the Collection Tube with the flow-through and place the Spin Column into a new Collection Tube.

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  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 on a new 1.5 ml tube.

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  11. Add 100  $\mu$ l Elution Buffer A and incubate at room temperature for 1 min. Note: The DNA may be eluted 2-times with 50  $\mu$ l of Elution Buffer A to increase the DNA yield.

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  12. Centrifuge at  $6000 \times g$  for 1 min. Note: The DNA can be eluted with a lower or a higher volume of Elution Buffer A (depending on the expected yield of genomic DNA). Elution with lower volumes of Elution Buffer A will increase the final DNA concentration. Store the extracted DNA at +4 °C or below -18 °C for long time storage.
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## **APPENDIX**

### *Limited Product Warranty*

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising from of the use, the results of use, or the inability to use this product.

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## RELATED PRODUCTS

### qPCR Kits for Food Contamination Testing

360-1025	Food Control™ qPCR <i>Salmonella enterica</i>	25 reactions
360-2025	Food Control™ qPCR <i>Yersinia enterocolitica</i>	25 reactions
360-3025	Food Control™ qPCR <i>Shigella spp.</i>	25 reactions
360-4025	Food Control™ qPCR <i>Campylobacter spp.</i>	25 reactions
360-5025	Food Control™ qPCR <i>Clostridium perfringens</i>	25 reactions
360-6025	Food Control™ qPCR <i>Shiga Toxin 1</i>	25 reactions
360-7025	Food Control™ qPCR <i>Shiga Toxin 2</i>	25 reactions
360-8025	Food Control™ qPCR <i>Escherichia coli O157</i>	25 reactions
360-9025	Food Control™ qPCR <i>Escherichia coli O104</i>	25 reactions
361-1025	Food Control™ qPCR <i>Listeria spp.</i>	25 reactions
361-2025	Food Control™ qPCR <i>Listeria monocytogenes</i>	25 reactions
361-3025	Food Control™ qPCR <i>Salmonella spp.</i>	25 reactions

### qPCR Kits for Vegan Control

370-2025/-2100	Vegan Control™	25/100 reactions
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### qPCR Kits for Meat Identification

370-1025/-1100	Meat ID™ Halal	25/100 reactions
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### qPCR Kits for Water Contamination Testing

33-2025/-2100/-2250	AquaScreen® <i>Legionella</i> species	25/100/250 reactions
34-2025/-2100/-2250	AquaScreen® <i>Legionella pneumophila</i>	25/100/250 reactions
34-6025/-6100/-6250	AquaScreen® <i>Pseudomonas aeruginosa</i>	25/100/250 reactions
34-7025/-7100/-7250	AquaScreen® <i>Escherichia coli</i>	25/100/250 reactions

### DNA Extraction kits

56-1010/1050/1200	Veno®GeM Sample Preparation Kit	10/50/200 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

### PCR Quantification Standards, 10<sup>8</sup> genomes / vial

52-0116	<i>Acholeplasma laidlawii</i>
52-0129	<i>Mycoplasma arginini</i>
52-0117	<i>Mycoplasma fermentans</i>
52-0115	<i>Mycoplasma gallisepticum</i>
52-0130	<i>Mycoplasma hyorhinis</i>
52-0112	<i>Mycoplasma orale</i>
52-0119	<i>Mycoplasma pneumoniae</i>
52-0103	<i>Mycoplasma salivarium</i>
52-0124	<i>Mycoplasma synoviae</i>
52-0164	<i>Spiroplasma citri</i>
52-5571	<i>Bordetella pertussis</i>
52-0083	<i>Escherichia coli</i>
52-0101	<i>Legionella pneumophila</i>
52-0071	<i>Pseudomonas aeruginosa</i>

See MB homepage for further available species

### Genomic DNA Extracts, 10±2 ng/vial

51-7058	<i>Salmonella enterica</i>
2140-04780	<i>Yersinia enterocolitica</i>
2137-04782	<i>Shigella flexneri</i>
2138-05570	<i>Shigella sonnei</i>
2102-04688	<i>Campylobacter jejuni</i>
2108-00756	<i>Clostridium perfringens</i>

51-0083 *Escherichia coli*  
 2115-08579 *Escherichia coli* O157:H  
 See Minerva homepage for further available species

**PCR Cycler Validation**

57-2102	PCR Cycler Check™ Advance	6 strips, 8 vials each
57-2103	PCR Cycler Check™ OneStep	100 reactions
57-2202	qPCR Cycler Check™	100 reactions

**Lab Monitoring Kits**

181-0010/-0050	SwabUp™ Lab Monitoring, For sample collection and DNA extraction	10/50 samples
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**PCR Mix**

191-0025/-0100/-0250	ConviFlex™ DNAmix, PCR Mix with Taq polymerase for conventional and qPCR	25/100/250 reactions
192-0025/-0100/-0250	ConviFlex™ RT-Taq Mix, RT-PCR Mix with Taq polymerase and retrotranscriptase for conventional and RT-qPCR	25/100/250 reactions

**PCR Clean™**

15-2025/-2200/-2500	DNA Decontamination Reagent, spray bottle/refill/canister	250 ml/4×500 ml/5 l
15-2001	DNA Decontamination Reagent, Wipes in dispenser box	50 wipes
15-2002	DNA Decontamination Reagent, Wipes, refill pack	5×50 wipes

**LabClean™**

15-4100	Molecular Microbiology Lab Cleaner	1 liter
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**Mycoplasma Off™**

15-1000/-5000	Surface Disinfectant Spray, spray bottle, refill canister	1 l/5 l
15-1001	Surface Disinfectant Wipes in dispenser box	50 wipes
15-5001	Surface Disinfectant Wipes in refill pack	5×50 wipes



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