ExtractNow™ Blood DNA Mini Kit

Direct isolation of genomic DNA from whole blood samples

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™ Blood DNA Mini kit is a very efficient tool for rapid isolation of genomic DNA from whole blood samples. The procedure is based on spin column purification and works with fresh or frozen blood as wells as with EDTA– or citrate-stabilized blood. Up to 400 µl of the blood sample can be used to obtain up to 30 µg of genomic DNA. The purified DNA is free of contaminants and suitable for many downstream applications such as PCR.

PRINCIPLE OF THE METHOD

The method is simple and consists of four general steps: (1) cell lysis, (2) selective binding of nucleic acids to 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 cell lysis and purification of genomic DNA in less than 30 minutes.

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 (18 to 25 °C). Before every use, ensure that all components have room temperature. Dissolve any precipitates in the solutions by moderate warming.

Kit component	10 extractions (602-1010)	50 extractions (602-1050)
Spin columns (red)	10 units	50 units
Collection tubes	50 units	5 x 50 units
Lysis Buffer E	12 ml	25 ml
Binding Buffer B	8 ml	50 ml
Wash Buffer B1	8 ml	30 ml
Wash Buffer B2	2 ml (add 18 ml of ethanol (>96 %) before first use)	10 ml (add 90 ml of ethanol (>96 %) before first use)
Elution Buffer A	2 x 2 ml	15 ml
Proteinase K	1 x 6 mg (add 0.3 ml of ddH_2O to each vial)	2 x 30 mg (add 1.5 ml of ddH_2O to each vial)

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™ Blood DNA Mini Prep kit contains reagents for isolating genomic DNA from blood samples. Additional consumables and equipment is supplied by the user:

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

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 overload spin columns. The normal volume of blood is 200 µl (see protocol 1) but 400 µl (see protocol 2) can be used as well.

PRECAUTIONS

The ExtractNow™ Blood DNA Mini 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.

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.

DO NOT add bleaching agents or acidic solutions directly to the sample preparation waste.

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 –20 °C. Repeated freeze/thaw cycles will reduce the enzyme activity. We therefore recommend to prepare aliquots.
- Do not use higher volumes of blood as defined in the protocols.
- Set up the heat block or thermomixer to 60 °C.
- Ensure that ethanol was added to Wash Buffer B2. 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.

ExtractNow™ Blood DNA Mini Kit

Included

E Lysis Buffer E
B Binding Buffer B
B1 Wash Buffer B1
B2 Wash Buffer B2
P Proteinase K
A Elution Buffer A

Duration

≤ 10 min

Additionally required

- Ethanol > 96 % abs.
- Bidest water
- 1.5 ml Reaction tubes
- Tools: Microcentrifuge
  Thermomixer
  Vortexer
  Pipettes + tips

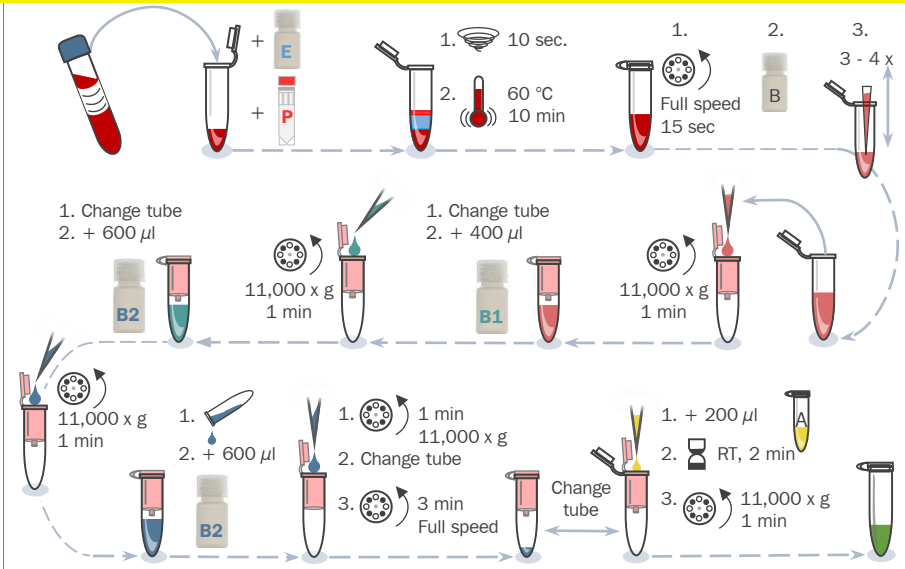
Before first use!

1. B2 + Ethanol > 96 % abs.
2. P + ddH_2O

Preparation

1. 60 °
2. A → A, 60 °

Procedure (DNA isolation from whole blood)



Storage

- Store the lyophilized Proteinase K at 4 °C and all other components at room temperature (18 to 25 °C).
- Store the extracted DNA at 4 °C or at –20 °C for long time storage.

Legend

- Blood
- Lysis Buffer E
- Proteinase K
- Wash Buffer B1
- Wash Buffer B2
- Elution Buffer
- Purified DNA
- Thermomixer
- Vortex
- Incubate
- Centrifuge

PROCEDURE

Protocol 1: DNA isolation from 200 µl whole blood samples

⇒ Before first use reconstitute Wash Buffer B2 with absolute ethanol as stated on the bottle label and rehydrate the Proteinase K with water.

⇒ Set the heat block or thermomixer to 60 °C.

⇒ Aliquot the needed volume of Elution Buffer A in a reaction tube and equilibrate at 60 °C.

1.1	Pipet 200 µl of the blood sample into a 1.5 ml reaction tube. Note: Adjust the volume of any sample that is below the 200 µl with PBS.
1.2	Add 200 µl Lysis Buffer E and 20 µl Proteinase K, mix vigorously by pulsed vortexing for 10 sec.
1.3	Incubate at 60 °C for 10 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. Optional: Add 4 µl RNase A (from stock solution 100 mg/ml; not included in the kit), vortex briefly and incubate at room temperature for 5 min.
1.4	Spin down the reaction tube for 15 sec to collect any condensate. Add 350 µl Binding Buffer B to the lysate, mix carefully by pipetting up and down (3 to 4 times; **Do not vortex!**) and pipet the mixture to a spin column (red) placed in a collection tube.
1.5	Centrifuge at 11,000 x g for 1 min. Note: If the solution has not completely passed through the spin column, centrifuge again at a higher speed or prolong the centrifugation.
1.6	Discard the collection tube and place the spin column in a new collection tube.
1.7	Add 400 µl Wash Buffer B1 and centrifuge at 11,000 x g for 1 min. Discard the collection tube and place the spin column in a new collection tube.
1.8	Add 600 µl Wash Buffer B2 and centrifuge at 11,000 x g for 1 min. Discard the flow through and re-assemble spin column and collection tube.
1.9	Repeat wash step with Wash Buffer B2. Discard the collection tube and place the spin column in a new collection tube.

1.10	Centrifuge at max. speed for 3 min to remove all traces of ethanol. Discard the collection tube and place the spin column in a new 1.5 ml tube.
1.11	Add 200 µl of pre-warmed Elution Buffer A and incubate at room temperature for 2 min.
1.12	Centrifuge at 11,000 x g for 1 min. A second elution step will increase the yield of extracted DNA. 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 at –20 °C for long time storage.

Protocol 2: DNA isolation from 400 µl whole blood samples

⇒ Before first use reconstitute Wash Buffer B2 with absolute ethanol as stated on the bottle label and rehydrate the Proteinase K with water.

⇒ Set the heat block or thermomixer to 60 °C.

⇒ Aliquot the needed volume of Elution Buffer A in a reaction tube and equilibrate at 60 °C.

2.1	Pipet 400 µl of the blood sample into a 2.0 ml reaction tube.
2.2	Add 400 µl Lysis Buffer E and 30 µl Proteinase K, mix vigorously by pulsed vortexing for 10 sec.
2.3	Incubate at 60 °C for 10 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. Optional: Add 4 µl RNase A (from stock solution 100 mg/ml; not included in the kit), vortex briefly and incubate at room temperature for 5 min.
2.4	Spin down the reaction tube for 15 sec to collect any condensate. Add 700 µl Binding Buffer B to the lysate, mix carefully by pipetting up and down (3 to 4 times; **Do not vortex!**) and pipet 750 µl of the sample to a spin column (red) placed in a collection tube.
2.5	Centrifuge at 11,000 x g for 1 min. Note: If the solution has not completely passed through the spin column, centrifuge again at a higher speed or prolong the centrifugation.
2.6	Discard the collection tube and place the spin column in a new collection tube. Pipet the remaining volume of the sample to the spin column and centrifuge at 11,000 x g for 1 min.
2.7	Discard the collection tube and place the spin column in a new collection tube.
2.8	Add 400 µl Wash Buffer B1 and centrifuge at 11,000 x g for 1 min. Discard the collection tube and place the spin column in a new collection tube.
2.9	Add 600 µl Wash Buffer B2 and centrifuge at 11,000 x g for 1 min. Discard the flow-through and re-assemble spin column and collection tube.
2.10	Repeat wash step with Wash Buffer B2. Discard the flow through and place the spin column in a new collection tube.

2.11	Centrifuge at max. speed for 3 min to remove all traces of ethanol. Discard the collection tube and place the spin column in a new 1.5 ml tube.
2.12	Add 200 µl of pre-warmed Elution Buffer A and incubate at room temperature for 2 min.
2.13	Centrifuge at 11,000 x g for 1 min. A second elution step will increase the yield of extracted DNA. 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 at –20 °C for long time storage.

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 the use, the results of use, or the inability to use this product.

ExtractNow™

The excellent way to isolate nucleic acid. System for quantitative detection of water pathogens. Kits for purifying nucleic acids from a variety of samples.
Find the optimized kit for your research needs.

ExtractNow™ Kit	Description	Package Size	Cat.-No.
DNA Mini Kit	Universally applicable DNA extraction method for a broad range of starting materials. Using a cutting-edge chemistry, the duration of the DNA purification is reduced to a minimum.	10 extractions 50 extractions	601-1010 601-1050
Blood DNA Mini Kit	Direct and rapid isolation of genomic DNA from whole blood up to 400 µl. High yields of up to 30 µg and extremely high-quality gDNA, depending on the sample and the amount used. There are two protocols available: < 200 µl and up to 400 µl blood samples Tested for EDTA and citrate stabilized and for fresh or frozen blood sample (including long time storage)	10 extractions 50 extractions	602-1010 602-1050
RNA Mini Kit	Purification of total RNA from eukaryotic and microbial materials. Prefiltration to selectively remove genomic DNA with no DNase digestion.	10 extractions 50 extractions	603-1010 603-1050
CleanUp Kit	Combination kit for fast extraction of DNA fragments from agarose gels or amplification products from PCR reaction mixtures. Flexible elution volumes between 30 and 50 µl and 10 to 20 µl. High recovery rates of up to 95 %. Capable of processing fragment lengths of up to 30 kb.	10 extractions 50 extractions	604-1010 604-1050
Plasmid Mini Kit	Easy and quick plasmid isolation from bacterial lysis.	10 extractions 50 extractions	605-1010 605-1050
Virus DNA/RNA Kit	Simultaneous isolation of viral DNA and RNA from a variety of starting materials. Extraction method based on the use of Spin Filters. Optimum removal of inhibitors ensures trouble-free use of nucleic acids in subsequent applications. Recommended for samples with unknown viruses. Includes Carrier Mix with internal DNA and RNA extraction control.	10 extractions 50 extractions	606-1010 606-1050

All kits for research use only. Not recommended for clinical applications.

RELATED PRODUCTS

Meat ID™

Identification of animal species
in meat and other foods by qPCR

Background

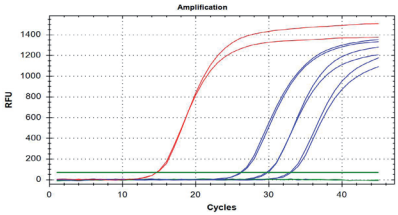
The identification of different meats in especially minced meat products is a serious task in food safety and ethical perspective, especially for muslims. Authentication of forbidden or none declared ingredients such as pork or substandard meat is essential to ensure confidence in the supply chain and regulatory compliance. Meat ID is available for rapid and reliable analysis from various matrices including raw, or even highly processed and cooked meat products where the DNA may be significantly degraded. It is possible to identify relevant species down to a threshold level of 0.5% with a semi-quantitative result.

Features

Principle	The assay is based on the TaqMan® principle and worked with FAM and HEX labled probes.
Target	The target sequence is a mitochondrial multi-copy gene (cytochrome b). Therefore, even very small amounts of DNA can lead to positive results.
Sensitivity	1 Genom Unit/PCR, ≙ 10 DNA copies/PCR
Content	Master Mix, freeze-dried Primer Probe Mix, freeze-dried Rehydration Buffer PCR Grade Water Internal Control Positiv Control
Sample Requirements	The DNA can be isolated from sample materials either by using an extraction kit designed to isolate gDNA e.g. ExtractNowTM DNA Mini Kit or by an in-house method.
Intended Use	For research only! Not for use in diagnostic procedures.
Time to Result	90 minutes
Storage	Components are maintainable at +2 to +8 °C. After rehydratisation the reagents must be stored at -18 °C.
Real Time Cycler	• qTOWER (Analytik Jena) • TOptical (Analytik Jena) • Rotor-Gene® Q (Qiagen GmbH) • LightCycler® (Roche Diagnostic GmbH) • Mastercycler® ep realplex (Eppendorf) • CFX ConnectTM (Bio-Rad) • Amplifa (Illumina ECO) • StepOnePlus™ (Applied Biosystems)

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

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

MB *Taq* DNA Polymerase

53-0050/0100/0200/0250	MB *Taq* DNA Polymerase (5 U/µl)	50/100/200/250 units
53-1050/1100/1200/1250	MB *Taq* DNA Polymerase (1 U/µl)	50/100/200/250 units

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

Mycoplasma Elimination

10-0200/0500/1000	Mynox® Mycoplasma Elimination Reagent	2/5/10 treatments
10-0201/0501/1001	Mynox® Gold Mycoplasma Elimination Reagent	2/5/10 treatments

PCR Quantification Standards, 1x10^8 genomes / vial

52-0112	*Mycoplasma orale*
52-0115	*Mycoplasma gallisepticum*
52-0116	*Acholeplasma laidlawii*
52-0117	*Mycoplasma fermentans*
52-0119	*Mycoplasma pneumonia*
52-0124	*Mycoplasma synoviae*
52-0129	*Mycoplasma arginini*
52-0130	*Mycoplasma hyorhinis*
52-0164	*Spiroplasma citri*

See Minerva homepage for further available species

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
15-2203	Wipes, single wrapped	30 wipes

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
15-1030	Wipes, single wrapped	30 sachets

ZellShield™

13-0050/0150	Contamination Prevention Reagent 100x concentrate	1000 ml/ 5 x 1000 ml

WaterShield™

15-3025/3075	Water Disinfection Additive for incubators and water baths 200x concentrate	30 x 5 ml/500 ml

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