ExtractNow™ DNA Mini Kit

Universally applicable DNA extraction method for a broad range of starting materials

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™ DNA Mini kit is a universal tool for rapid and efficient isolation of genomic DNA from various sources such as tissue (including paraffin-embedded tissue), buccal swabs, and cells. The procedure is based on spin column purification. Up to 50 mg of starting material can be used to obtain up to 60 μg of 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) tissue homogenisation and 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. After tissue homogenisation, the kit's chemistry facilitates fast purification of genomic DNA in less than 10 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 (601-1010)	50 extractions (601-1050)
Spin columns (blue)	10 units	50 units
Collection tubes	20 units	2 x 50 units
Lysis Buffer C	10 ml	25 ml
Binding Buffer C	16 ml	16 ml
Wash Buffer E	6 ml (add 14 ml ethanol (>96%) before first use)	24 ml (add 56 ml ethanol (>96%) before first use)
Elution Buffer A	2 x 2 ml	25 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™ DNA Mini kit contains reagents for isolating DNA from various sources. 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
- Xylen or Octan (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 overload spin columns. The maximum amounts of starting material are:

- up to 40 mg of tissue samples (including paraffin-embedded samples)
- 0.5 to 1 cm in length for rodent tail samples
- up to $5x10^6$ eukaryotic cells

PRECAUTIONS

The ExtractNow™ 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.

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

IMPORTANT NOTES

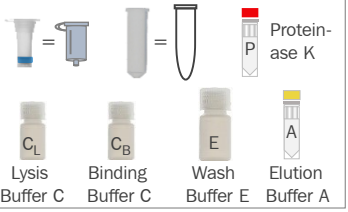
- Dissolve the Proteinase K with the given volume of ddH_2O and mix thoroughly by pipetting. Dissolved Proteinase K must be stored at –20 °C. Repeated freeze/thaw cycles will reduce the enzyme activity. We therefore recommend to prepare aliquots.
- Set up the heat block to 50 °C.
- Ensure that ethanol was added to Wash Buffer E. Do not use other alcohol apart from ethanol as it will lead to inconsistent yields.
- Ensure that water was added to proteinase K.
- 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™ DNA Mini Kit

Included



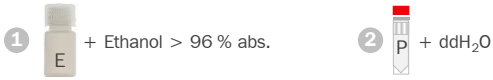
Duration

≤ 10 min

Additionally required

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

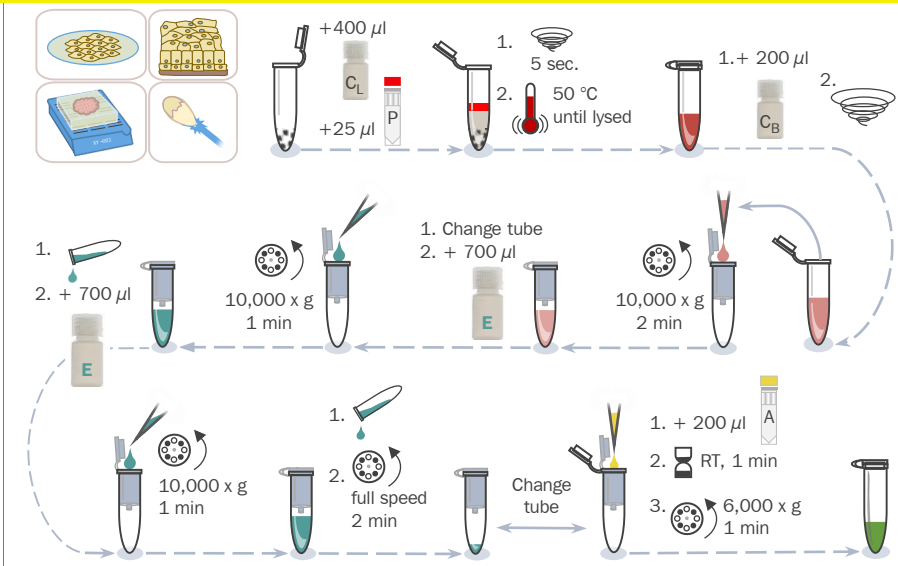
Before first use!



Preparation

50 °C / 90°C

Procedure (Overview only! For detailed information see protocols on following pages.)



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

- Raw material
- Proteinase K
- Lysis Buffer
- Lysed Sample
- Wash Buffer
- Elution Buffer
- Purified DNA
- Thermomixer
- Vortex
- Incubate
- Centrifuge

PROCEDURE

Protocol 1: DNA isolation from tissues including rodent tails (see protocol 2 for paraffin-embedded tissue)

⇒ Before first use reconstitute Wash Buffer E with absolute ethanol
⇒ Rehydrate the proteinase K with water.
⇒ Set the heat block to 50 °C.

1.1	Cut up to 40 mg of the tissue into small pieces and place the sample in a 1.5 ml reaction tube.
1.2	Add 400 µl Lysis Buffer C and 25 µl Proteinase K, mix vigorously by pulsed vortexing for 5 sec. Optional: Add 3 µl RNase A (from stock solution 100 mg/ml; not included in the kit) to remove the RNA.
1.3	Incubate at 50 °C until the sample is completely lysed (takes up to 2 hours for most tissues or ~3 hours for rodent tails). 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. Note: The incubation must be stopped when the tissue is completely dissociated as further incubation will be detrimental for DNA integrity.
1.4	Centrifuge the tube at 10,000 x g for 30 sec to pellet any unlysed material. Transfer the supernatant into a new 1.5 ml tube.
1.5	Add 200 µl Binding Buffer C to the lysate, mix by vortexing or by pipetting up and down several times. Note: It is important that the sample and Binding Buffer C are mixed vigorously to obtain a homogeneous solution.
1.6	Pipet the sample to a spin column placed in a collection tube. Close the cap and 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.
1.7	Discard the collection tube and place the spin column in a new collection tube.
1.8	Add 700 µl Wash Buffer E and centrifuge at 10,000 x g for 1 min. Discard the flow-through and re-assemble spin column and collection tube.

1.9	Repeat wash step once more. Discard the flow-through and re-assemble spin column and collection tube.
1.10	Centrifuge at max. speed for 2 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 Elution Buffer A and incubate at room temperature for 1 min.
1.12	Centrifuge at 6000 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 paraffin-embedded tissues

⇒ Before first use reconstitute Wash Buffer E with absolute ethanol
⇒ Rehydrate the proteinase K with water.
⇒ Set the heat block to 50 °C and 90 °C.

2.1	Place the piece of paraffin-embedded tissue in a 2.0 ml reaction tube. Add 1 ml of Xylene or Octane and vortex gently to dissolve the paraffin until the sample is transparent (paraffin remains white).
2.2	Centrifuge at max. speed for 5 min. Discard the supernatant carefully by aspirating with a pipet. Do not remove the pellet. Note: This step should be repeated if any paraffin is still in the sample.
2.3	Add 1 ml ethanol (≥96 %) to the pellet and vortex vigorously. Centrifuge at maximum speed for 3 min.
2.4	Remove the ethanol by pipetting. Do not remove the pellet. Repeat the wash step with ethanol once more. Incubate the open tube at 37 °C for 10 – 15 min to completely evaporate any residual ethanol.
2.5	Add 400 µl Lysis Buffer C and 25 µl Proteinase K, mix vigorously by pulsed vortexing for 5 sec.
2.6	Incubate at 50 °C until the sample is completely lysed. After lysis step place the sample into a thermomixer pre-warmed at 90 °C and incubate the sample for 1 hour. Important: Do not place the sample in the thermomixer before the temperature is at 90 °C.
2.7	Add 200 µl Binding Buffer C to the lysate, mix by vortexing or by pipetting up and down several times. Note: It is important that the sample and Binding Buffer C are mixed vigorously to obtain a homogeneous solution.
2.8	Pipet the sample to a spin column placed in a collection tube. Close the cap and 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.
2.9	Discard the collection tube and place the spin column in a new collection tube.

2.10	Add 700 µl Wash Buffer E and centrifuge at 10,000 x g for 1 min. Discard the flow-through and re-assemble spin column and collection tube.
2.11	Repeat wash step once more. Discard the flow-through and re-assemble spin column and collection tube.
2.12	Centrifuge at max. speed for 2 min to remove all traces of ethanol. Discard the collection tube and place the spin column in a new 1.5 ml tube.
2.13	Add 200 µl Elution Buffer A and incubate at room temperature for 1 min.
2.14	Centrifuge at 6000 x g for 1 min. A second elution step will increase the yield of the 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 3: DNA isolation from buccal swabs

⇒ Before first use reconstitute Wash Buffer E with absolute ethanol
⇒ Rehydrate the proteinase K with water.
⇒ Set the heat block to 50 °C.

3.1	Place the swab in a 1.5 ml reaction tube. Important: To get a maximum DNA yield it is essential to leave the swab during the complete lysis time within the reaction tube. We recommend to cut off the shaft to ensure that the tube can be capped. An early removal of the swab from the tube will lead to reduced final yield.
3.2	Add 400 µl Lysis Buffer C and 25 µl Proteinase K, mix vigorously by pulsed vortexing for 5 sec. Optional: Add 3 µl RNase A (from stock solution 100 mg/ml; not included in the kit) to remove the RNA.
3.3	Incubate at 50 °C for 10 to 15 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.
3.4	Remove the swab from the tube and squeeze the swab on the wall of the tube to remove all liquid from the swab.
3.5	Add 200 µl Binding Buffer C to the lysate, mix by vortexing or by pipetting up and down several times. Note: It is important that the sample and Binding Buffer C are mixed vigorously to obtain a homogeneous solution.
3.6	Pipet the sample to a spin column placed in a collection tube. Close the cap and 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.
3.7	Discard the collection tube and place the spin column in a new collection tube.
3.8	Add 700 µl Wash Buffer E and centrifuge at 10,000 x g for 1 min. Discard the flow-through and re-assemble spin column and collection tube.
3.9	Repeat wash step once more. Discard the flow-through and re-assemble spin column and collection tube.

3.10	Centrifuge at max. speed for 2 min to remove all traces of ethanol. Discard the collection tube and place the spin column in a new 1.5 ml tube.
3.11	Add 200 µl Elution Buffer A and incubate at room temperature for 1 min.
3.12	Centrifuge at 6000 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 4: DNA isolation from cell cultures (up to 5 x 10^6 cells)

⇒ Before first use reconstitute Wash Buffer E with absolute ethanol
⇒ Rehydrate the proteinase K with water.
⇒ Set the heat block to 50 °C.

4.1	Transfer your cells (max. 5 x 10^6 cells) in a 1.5 or 2.0 ml reaction tube. Pellet cells by centrifugation at 5000 x g for 10 min. Discard the supernatant.
4.2	Add 400 µl Lysis Buffer C and 25 µl Proteinase K, mix vigorously by pulsed vortexing for 5 sec. Optional: Add 3 µl RNase A (from stock solution 100 mg/ml; not included in the kit) to remove the RNA.
4.3	Incubate at 50 °C until the sample is completely lysed (~30 min). We recommend the use of a thermomixer for a permanent shaking of the sample as it will increase the DNA yield. Alternatively, vortex the samples 3 to 4 times during the incubation.
4.4	Add 200 µl Binding Buffer C to the lysate, mix by vortexing or by pipetting up and down several times. Note: It is important that the sample and Binding Buffer C are mixed vigorously to obtain a homogeneous solution.
4.5	Pipet the sample to a spin column placed in a collection tube. Close the cap and 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.
4.6	Discard the collection tube and place the spin column in a new collection tube.
4.7	Add 700 µl Wash Buffer E and centrifuge at 10,000 x g for 1 min. Discard the flow-through and re-assemble spin column and collection tube.
4.8	Repeat wash step once more. Discard the flow-through and re-assemble spin column and collection tube.
4.9	Centrifuge at max. speed for 2 min to remove all traces of ethanol. Discard the collection tube and place the spin column in a new 1.5 ml tube.

4.10	Add 200 µl Elution Buffer A and incubate at room temperature for 1 min.
4.11	Centrifuge at 6000 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

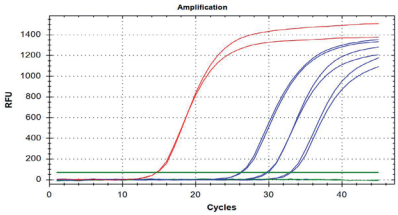
The identification of different meats in especially minced meat products is a serious task in food safety and ethical perspective, especially for muslims. Authentication of forbidden or none declared ingredients such as pork or substandard meat is essential to ensure confidence in the supply chain and 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)

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