INSTRUCTIONS FOR USE

Rons Tissue & Blood DNA Mini Kit

Kit for the isolation and purification of genomic DNA from blood, tissue and cultured cells.

For Research Use Only (RUO)

List of Components	Quantity	
	REF 803496	REF 803496L
	50 preps	5 × 50 preps
Mini Spin Columns	50 pcs	5 × 50 pcs
Collection Tubes 2.0 ml	50 pcs	5 × 50 pcs
Lysis Buffer TCBL-1	11 ml	5 × 11 ml
Binding Buffer TGP-2	11 ml	5 × 11 ml
Wash Buffer TGI-3 concentrate	18 ml	5 × 18 ml
Wash Buffer TE-4 concentrate	5.1 ml	5 × 5.1 ml
Elution Buffer EL-3	11 ml	5 × 11 ml
Proteinase K (20 mg/ml)	1 ml	5 × 1 ml
Manual	1 pc	1 pc

IFU valid from	**November 2026**
IFU reviewed	**November 2026**

BIORON GmbH – In den Rauhweiden 20 – 67354 Römerberg - Germany
Phone:+49623229845-0 - Fax:+49623229845-29
info@bioron.net www.bioron.net

Introduction

Rons Tissue & Blood DNA Mini Kit is designed for the isolation and purification of high-quality genomic DNA from human and animal biological samples. The kit combines silica membrane spin column technology with optimized lysis, binding, washing, and elution buffers to enable efficient DNA recovery while minimizing the carryover of proteins, inhibitors, and other contaminants.

The kit is suitable for the processing of a broad range of sample materials, including fresh or frozen whole blood collected with or without anticoagulants such as EDTA or citrate, mammalian tissue samples, preserved tissue specimens, paraffin-embedded tissues, mouse tails, and cultured cells. Isolation of leukocytes prior to extraction is not required.

The streamlined workflow enables parallel processing of multiple samples with minimal risk of cross-contamination. Purified DNA is suitable for immediate downstream molecular biology applications or can be stored for later use under appropriate conditions. The product is intended for use by personnel trained in molecular biology techniques.

Applications

The purified genomic DNA obtained with the kit is suitable for a variety of molecular biology applications, including:

- Conventional PCR
- Real-time PCR (qPCR)
- DNA sequencing
- Restriction enzyme digestion
- Genotyping analyses
- Gel electrophoresis
- Cloning workflows
- General downstream genomic DNA applications

The kit is suitable for the extraction of genomic DNA from:

- Fresh or frozen whole blood
- Blood collected with EDTA or citrate anticoagulants
- Mammalian tissue samples (e.g., muscle, brain)
- Alcohol-preserved tissue
- Paraffin-embedded tissue sections
- Mouse tails
- Adherent and suspension cell cultures

Principle of the method

The purification procedure is based on selective adsorption of genomic DNA to a silica membrane under optimized chaotropic salt conditions.

Samples are first lysed using Lysis Buffer TCBL-1 in combination with Proteinase K, resulting in efficient disruption of cellular structures and digestion of proteins. Following lysis, Binding Buffer TGP-2 is added to create conditions that promote DNA binding to the silica membrane of the spin column.

After application of the lysate to the spin column, contaminants such as proteins, lipids, salts, and other cellular components are removed through sequential washing steps using Wash Buffer TGI-3

and Wash Buffer TE-4. Residual wash buffer is eliminated by centrifugation and drying of the membrane. Finally, purified genomic DNA is recovered by elution with Elution Buffer EL-3.

The resulting DNA is of high purity and suitable for a wide range of downstream molecular biology applications.

Product Specifications

Parameter	Specification
Product Name	Rons Tissue & Blood DNA Mini Kit
Product Number	803496 / 803496L
Technology	Silica membrane spin-column purification
Target Molecule	Genomic DNA (gDNA)
Sample Types	Whole blood, EDTA blood, citrate blood, mammalian tissue, alcohol-preserved tissue, paraffin-embedded tissue, mouse tails, cultured cells
Maximum Blood Input	Up to 200 µL whole blood
Maximum Tissue Input	Up to 200 mg tissue (depending on tissue type)
Maximum Cell Input	Up to 1 × 10^7 cultured cells
Lysis Method	Chemical lysis combined with Proteinase K digestion
DNA Binding Principle	Selective adsorption of DNA to a silica membrane under chaotropic salt conditions
Purification Format	Spin-column based purification
Elution Buffer	EL-3 Buffer
DNA Type Recovered	High-quality genomic DNA
Downstream Applications	PCR, qPCR, DNA sequencing, restriction digestion, genotyping, cloning and other molecular biology applications
Processing Time	Typical hands-on time: approximately 30 min. The total processing time depends on sample type and lysis requirements.
Storage Conditions	See component-specific storage requirements
Intended User	Professional laboratory personnel trained in molecular biology techniques
Product Classification	Research Use Only (RUO)
Elution Volume	50-100 µL
DNA Purity	Typical A260/A280 ratio 1.7–2.0
Shelf Life	24 months from date of manufacture
Functional Quality Control	Every production lot is tested for genomic DNA extraction performance

Product use limitations

This product is intended exclusively for Research Use Only (RUO). It is designed for research and laboratory applications performed by qualified personnel trained in molecular biological techniques.

The kit is not intended for diagnostic procedures, therapeutic applications, patient management, drug testing, or any other clinical use involving humans. Results obtained with this product must not be used as the sole basis for clinical or diagnostic decisions.

Users are responsible for determining the suitability of the product for their specific application and experimental requirements.

Warning and precautions

For professional laboratory use only. Appropriate laboratory safety practices should be followed at all times.

1. General Precautions

- Wear suitable laboratory clothing, protective gloves, and safety glasses during handling.
- Do not pipette by mouth.
- Avoid contact of reagents with skin, eyes, or clothing.
- Handle all biological samples as potentially infectious material.
- Consult the relevant Safety Data Sheets before use.

2. Hazardous Components

The kit contains reagents classified as hazardous, including:

- Lysis Buffer TCBL-1 containing sodium dodecyl sulfate (SDS).
- Binding Buffer TGP-2 containing guanidine hydrochloride.
- Wash Buffer TGI-3 containing guanidine hydrochloride.
- Proteinase K Solution.

3. Handling Recommendations

- Avoid inhalation of aerosols and vapors.
- Prevent contact with eyes and skin.
- Use only in accordance with established laboratory procedures.
- Dispose of reagents and biological materials according to applicable local regulations.
- Use sterile consumables and process samples promptly to minimize nucleic acid degradation.

4. Technical Notes

- If precipitates are present in Lysis Buffer TCBL-1, dissolve them by warming the buffer to approximately 55°C before use.
- Prior to the first extraction, prepare Wash Buffer TGI-3 and Wash Buffer TE-4 by adding the required volumes of alcohol as indicated on the bottle labels.
- Complete removal of wash buffer residues is essential, as residual alcohol may reduce DNA recovery and affect downstream performance.

Transportation & Storage

All kit components should be transported and stored according to the conditions specified below to ensure optimal performance throughout the product shelf life.

Component	Storage Conditions
All buffers and other components including Spin Columns Collection Tubes Buffers	18 °C to 25 °C
Proteinase K Solution	-18 °C to -22 °C

1. Storage Recommendation

- Store all components protected from direct light.
- Keep buffer bottles tightly closed and in an upright position.
- Remove only the number of spin columns required for immediate use; remaining columns should be kept in the original sealed packaging.
- Product performance is guaranteed only when the recommended storage conditions are maintained.

2. Storage of Purified DNA

Purified genomic DNA may be used immediately for downstream applications or stored at -18 °C to -22 °C for long-term preservation.

Handling Recommendations

1. General Handling Recommendations

- Before starting the procedure, ensure that all kit components, have been stored according to the recommended conditions and have reached room temperature (except Proteinase K).

- Wear appropriate personal protective equipment (PPE), including laboratory coat, gloves, and safety glasses.
- Use sterile, nuclease-free consumables and pipette tips.
- Handle biological samples according to established laboratory safety procedures.
- Mix reagents thoroughly before use and briefly centrifuge if necessary.
- Prepare Wash Buffers TGI-3 and TE-4 with the required volume of alcohol before first use.
- If precipitates are visible in Lysis Buffer TCBL-1, dissolve them by incubation at approximately 55 °C.
- Avoid cross-contamination by changing pipette tips between samples.
- Follow the recommended sample input amounts specified for each sample type.

2. Procedure Overview

This overview is intended as a quick reference only. For a graphical representation of the workflow, see Section **Detailed Protocol 4. Flowchart of the Extraction** (page 8). For complete operating instructions, see Section **Detailed Protocol 5. Genomic DNA Purification Procedure** (page 10).

The purification workflow consists of the following steps:

Step 1. Sample Preparation and Lysis

Samples are homogenized where required and lysed using Lysis Buffer TCBL-1 and Proteinase K to release genomic DNA.

Step 2. DNA Binding

Binding Buffer TGP-2 is added to create conditions that promote selective binding of DNA to the silica membrane of the spin column.

Step 3. Column Washing

Bound DNA is purified through sequential washing steps with Wash Buffers TGI-3 and TE-4 to remove salts, proteins, and other contaminants.

Step 4. Membrane Drying

Additional centrifugation removes residual wash buffer and ethanol, ensuring optimal downstream performance.

Step 5. DNA Elution

Purified genomic DNA is recovered using Elution Buffer EL-3 and is ready for immediate downstream applications or storage.

3. Important Notes

- Complete removal of residual ethanol is essential, as carryover may inhibit downstream enzymatic reactions.
- DNA yield and purity may vary depending on sample type, sample quality, storage conditions, and handling.
- For optimal performance, process samples as soon as possible after collection.
- Purified DNA should be stored at the recommended temperature if not used immediately.

Detailed Protocol

1. Materials required but not provided

- 96 - 100 % Ethanol
- 100 % Isopropanol
- Microcentrifuge
- Vortex-Mixer
- Heat block, incubator, water bath, or thermomixer capable of 55 °C and 70 °C
- Sterile 1.5 mL microcentrifuge tubes
- Pipettes and sterile filter tips

2. Preparation of Wash Buffers

Before first use, prepare the wash buffers according to the table below:

Buffer	Preparation
Wash Buffer TGI-3	Add 7.6 ml Isopropanol to 18 ml concentrate
Wash Buffer TE-4	Add 21 ml Ethanol to 5.1 ml concentrate

Mix thoroughly after alcohol addition and mark the bottles accordingly.

Important Notes

- If precipitates are visible in Lysis Buffer TCBL-1, incubate the buffer at approximately 55 °C until completely dissolved.
- Preheat Elution Buffer EL-3 to 55 °C to 70 °C before elution for optimal DNA recovery.
- Equilibrate all reagents except Proteinase K to room temperature before use.

3. Sample Preparation

- **Tissue Samples**

Fresh tissue should be processed immediately after collection or stored frozen until use.

Sample Type	Maximum Input
Fresh tissue	Up to 200 mg
Brain tissue	Up to 50 mg
Alcohol-preserved tissue	Up to 50 mg
Paraffin-embedded tissue	Sections up to 10 µm thickness or up to 50 mg
Mouse tail	Up to 1 cm

Cut tissue into small pieces and homogenize if required.

- **Blood Samples**

Use up to 200 µL whole blood.

Suitable sample types include:

- Fresh blood
- Frozen blood
- EDTA blood
- Citrate blood

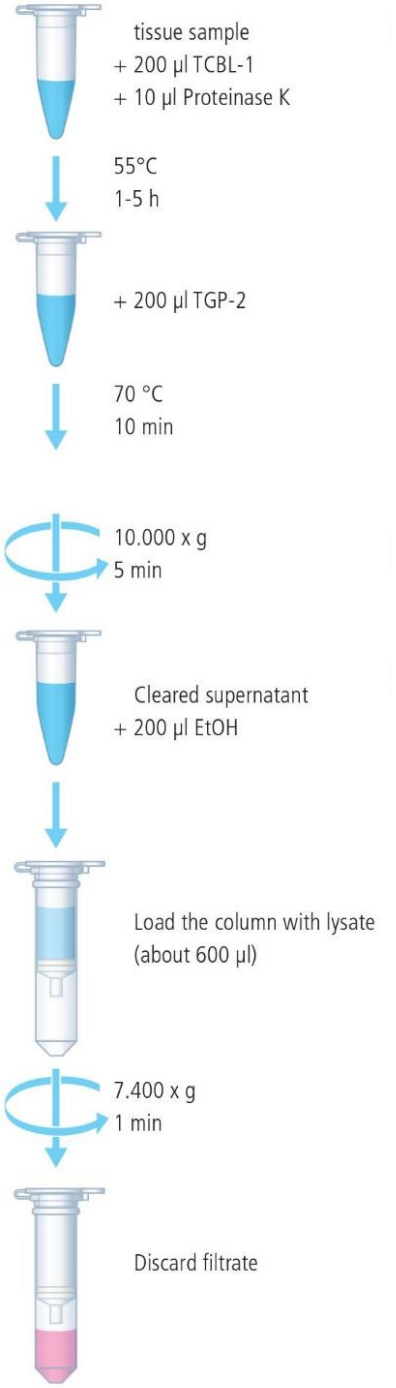
No prior leukocyte separation is required.

- **Cultured Cells**

Use up to 1 × 10^7 cells.

Pellet cells prior to extraction and remove the supernatant completely.

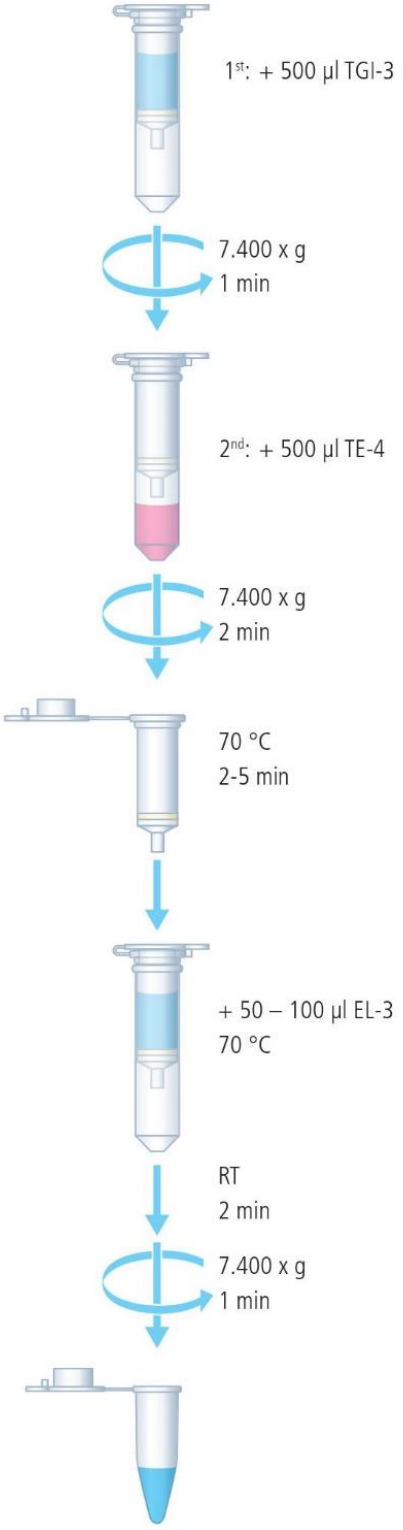
4. Flowchart of the Extraction



Step 1. Sample Preparation and Lysis

Step 2. DNA Binding

BIORON GmbH – In den Rauhweiden 20 – 67354 Römerberg - Germany
Phone:+49623229845-0 - Fax:+49623229845-29
info@bioron.net www.bioron.net



Step 3. Column Washing

Step 4. Membrane Drying

Step 5. DNA Elution

Figure 1: DNA Extraction Workflow Overview.

BIORON GmbH – In den Rauhweiden 20 – 67354 Römerberg - Germany
Phone:+49623229845-0 - Fax:+49623229845-29
info@bioron.net www.bioron.net

5. Genomic DNA Purification Procedure

Step 1. Sample Lysis

Transfer the prepared sample to a sterile 1.5 mL microcentrifuge tube.
Add:

- 200 µL Lysis Buffer TCBL-1
- 10 µL Proteinase K Solution

Vortex vigorously for approximately 1 minute.
Incubate at 55 °C until complete sample digestion is achieved. Depending on sample type, incubation may require 1 hour up to overnight.

Step 2. DNA Binding Preparation

Add:

- 200 µL Binding Buffer TGP-2

Mix thoroughly by vortexing for approximately 10 seconds.
Incubate at 70 °C for 10 minutes.
Centrifuge at 10,000 × g for 5 minutes.
Carefully transfer the clear supernatant to a new tube.
Add:

- 200 µL Ethanol (96 - 100 %)

Mix thoroughly by vortexing.

DNA Binding to the Spin Column

Place a spin column into a collection tube.
Load the complete lysate (approximately 600 µL) onto the column.
Centrifuge at 7,400 × g for 1 minute.
Discard the flow-through and place the column back into the collection tube.

Step 3. First Column Washing

Add:

- 500 µL Wash Buffer TGI-3

Centrifuge at 7,400 × g for 1 minute.
Discard the flow-through.

Second Column Washing

Add:

- 500 µL Wash Buffer TE-4

Centrifuge at 7,400 × g for 2 minutes.
Discard the flow-through.

Step 4. Membrane Drying

Transfer the spin column to a clean 1.5 mL microcentrifuge tube.
Incubate the column at 70 °C for 2-5 minutes to remove residual ethanol.

Important: Complete removal of residual alcohol is essential, as ethanol carry-over can reduce DNA recovery and negatively affect downstream enzymatic reactions.

Step 5. DNA Elution

Place the spin column into a new, labelled 1.5 mL microcentrifuge tube (not provided).
Add:

- 50 - 100 µL preheated Elution Buffer EL-3 (55 °C – 70 °C)

Pipette the buffer directly onto the center of the membrane.
Incubate for 2 minutes at room temperature.
Centrifuge at 7,400 × g for 1 minute.
Collect the eluate containing purified genomic DNA.

6. Storage of Purified DNA

Purified DNA may be used immediately for downstream applications or stored at −18 °C to −22 °C for long-term storage

7. Expected Downstream Applications

Purified genomic DNA is suitable for:

- Conventional PCR
- Real-time PCR (qPCR)
- DNA sequencing
- Restriction enzyme digestion
- Genotyping
- Cloning applications
- Electrophoretic analysis

Troubleshooting

Observation	Possible Cause	Recommendation
Low or no DNA yield	Incomplete sample lysis	Ensure sufficient homogenization and complete Proteinase K digestion. Extend incubation time if required.
	Lysis Buffer TCBL-1 not added	Verify that Lysis Buffer TCBL-1 was added and thoroughly mixed with the sample.
	Binding Buffer TGP-2 not added	Verify that Binding Buffer TGP-2 was added and thoroughly mixed prior to column loading.
	Proteinase K inactive or improperly stored	Store Proteinase K at -18 °C to -22 °C. Avoid repeated freeze-thaw cycles.
Spin column clogging or slow flow-through	Excess sample material	Do not exceed the recommended sample input amounts.
	Incomplete tissue homogenization	Ensure complete disruption and digestion of the sample before loading onto the column.

Observation	Possible Cause	Recommendation
DNA degradation or DNA smear	Nuclease contamination	Use fresh samples whenever possible and process promptly. Use nuclease-free consumables.
Low A260/A280 ratio	Protein carry-over	Ensure complete lysis and perform all washing steps according to the protocol.
Low downstream PCR performance	Ethanol carry-over	Ensure complete removal of wash buffer and extend the membrane drying step if necessary.
	Salt carry-over	Verify that all recommended washing steps have been performed.

Symbol	Meaning	Symbol	Meaning
REF	Catalogue number	LOT	Batch code
	Temperature limit		Follow the instructions for use
	Manufacturer		Use-by date
	Date of manufacture		Contains sufficient for <n> tests
	Caution		Keep away from sunlight

BIORON GmbH – In den Rauhweiden 20 – 67354 Römerberg - Germany
Phone:+49623229845-0 - Fax:+49623229845-29
info@bioron.net www.bioron.net