



Ron's Tissue & Blood DNA Mini Kit

Instructions for Use

Ron's Tissue & Blood DNA Mini Kit

Kit for the isolation of genomic DNA from human and animal tissue incl. mouse tail and preserved tissue

GLU (General Lab Use)

Ron's Tissue & Blood DNA Mini Kit	REF 803496 (50 preps)
	REF 803496L (5 x 50 preps)
Valid from:	February 2024

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Ron's Tissue & Blood DNA Mini Kit

1. Introduction

The Ron's Tissue & Blood DNA Mini Kit is designed for extraction of DNA from tissue and blood from animal or human origin.

The kit contains spin columns, Proteinase K and optimized buffers for easy and timesaving high yield DNA purification and efficient removal of contaminants.

This Kit is suitable for DNA isolation from fresh or frozen blood - with or without coagulating reagents like EDTA or citrate. A prior separation of the leukocytes is not necessary. It can be used for adherent cell suspensions and all different kinds of mammalian tissue (e.g. muscle or brain). The tissue can be fresh or frozen, but also alcohol-preserved or even paraffin-embedded.

The protocol is suitable for processing multiple samples simultaneously without risk of cross contamination. The isolated DNA can directly be used in PCR or other applications or stored at - 18 °C to - 22 °C until later use.

The product should only be used by experts trained in the use of molecular biology methods.

2. Content of the Kit

Content	S803496 10 preps (sample size)	803496 50 preps	803496L 5 x 50 preps	Storage
Mini Spin Columns	10	50	5 x 50	RT
Collection Tubes 2.0 ml	10	50	5 x 50	RT
Lysis Buffer TCBL-1 *	11 ml	11 ml	5 x 11 ml	RT
Binding Buffer TGP-2	11 ml	11 ml	5 x 11 ml	RT
Wash Buffer TGI-3 concentrate **	18 ml	18 ml	5 x 18 ml	RT
Wash Buffer TE-4 concentrate **	5.1 ml	5.1 ml	5 x 5.1 ml	RT
Elution Buffer EL-3	11 ml	11 ml	5 x 11 ml	RT
Proteinase K (20 mg/ml)	1 ml	1 ml	5 x 1 ml	-20°C
Manual	1	1	1	-

* Precipitates in Lysis Buffer TCBL-1 should be dissolved by warming up to 55 °C.

** Please see page 5 for preparation of Wash buffer TGI-3 and Wash Buffer TE-4 before starting the first extraction.

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3. Storage Conditions and Stability

Proteinase K is delivered as solution and should be stored upon arrival at - 18 °C to - 22 °C. Spin columns are packed in a closed bag, please only remove the quantity currently needed. Spin columns, Collection tubes and buffers should be stored at room temperature (18 - 25 °C). All components should be protected from light. Make sure buffer bottles are stored upright. Guarantee for full performance of the kit as specified in this manual is only valid if storage conditions are followed.

4. Quality Control & Specification

The performance of the **Ron's Tissue & Blood DNA Mini Kit** is monitored routinely on a lot-to-lot basis. The efficiency of the DNA extraction equals 100 %. It was determined on different samples from tissue and blood with gDNA content of 100 copies in a sample, prepared from a positive standard reference sample.

5. Safety Information

The following components of **Ron's Tissue & Blood DNA Mini Kit** contain hazardous contents. It is strongly recommended to wear a lab coat, disposable gloves and protective goggles when working with chemicals. More detailed information is available in the material safety data sheet (MSDS), which can be requested from the manufacturer.

Component	Hazard content	GHS symbol		Hazard phrases	Precaution phrases
Lysis Buffer TCB-1	SDS Sodium dodecyl sulphate (0.1-1%)		Warning	302, 319	280, 301+312 305+351+338, 330, 337+313
Binding Buffer TGP-2	Guanidine hydrochloride 36-50%		Warning	302, 319	280, 301+312 305+351+338, 330, 337+313
Wash Buffer TGI-3	Guanidine hydrochloride 36-50%		Warning	302, 319	280, 301+312 305+351+338, 330, 337+313
Proteinase K Solution	Proteinase K 20 mg/ml	 	Warning Danger	315, 319, 334, 335	261,280, 302+352, 304+340, 305+351+338, 312,332+313, 337+313, 342+311, 403+233

Ron's Tissue & Blood DNA Mini Kit**6. Protocol****6.1 Additional Material Required**

- 96-100 % ethanol
- 100 % isopropanol
- Incubator/ heat shaker or water bath
- Microcentrifuge
- Receiver tubes (1.5 ml)

6.2 Before starting the first extraction

Wash Buffer TGI-3 and Wash Buffer TE-4 are concentrates.

Before using for the first time, add the appropriate amount of isopropanol and ethanol as noted at the table below:

Component	
Wash Buffer TGI-3	Add 7.6 ml isopropanol to 18 ml
Wash Buffer TE-4	Add 21 ml ethanol to 5.1 ml

Add the required amount of alcohol and mix well.

Mark the bottles on the label that the alcohols have been added.

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6.3 Sample preparation

To avoid DNA degradation, fresh tissue should be kept chilled while cut into small pieces or homogenized with a blender. Grinding of tissue frozen under liquid nitrogen is an alternative method for homogenization. Small pieces of tissue incl. mouse tails can be used directly for DNA extraction. Material should be processed immediately for DNA extraction.

Fresh tissue: For homogenization, cut up to **200 mg fresh tissue** or up to **50 mg brain** into small pieces and homogenize with a blender. Take care to keep material chilled during the procedure or freeze immediately at -20 °C to avoid DNA degradation.

Preserved tissue: Cut **50 mg alcohol-preserved tissue** into small pieces and extract DNA as below. Highest yields of paraffin-embedded tissues are obtained, when the sample is cut with a microtome to up to 10 µm thickness. Alternatively use up to 50 mg material and slice.

Mouse tail: Up to **1 cm mouse tail** can be used. Transfer homogenized or cut material in a sterile 1.5 ml reaction tube (not provided).

Blood: Use 200 µl of the suspended blood sample. For isolation of mononuclear cells (lymphocytes and monocytes) from blood please use lymphocyte separation media (iso-osmotic polysucrose and diatrizoate solution with density of 1.077 g/l). After separation by centrifugation, the layer with cells can be isolated and washed with PBS. Transfer 200 µl of cells and isolate Genomic DNA according to the protocol (see below).

Cells: suspensions up to 10⁷ adherent cells can be used.

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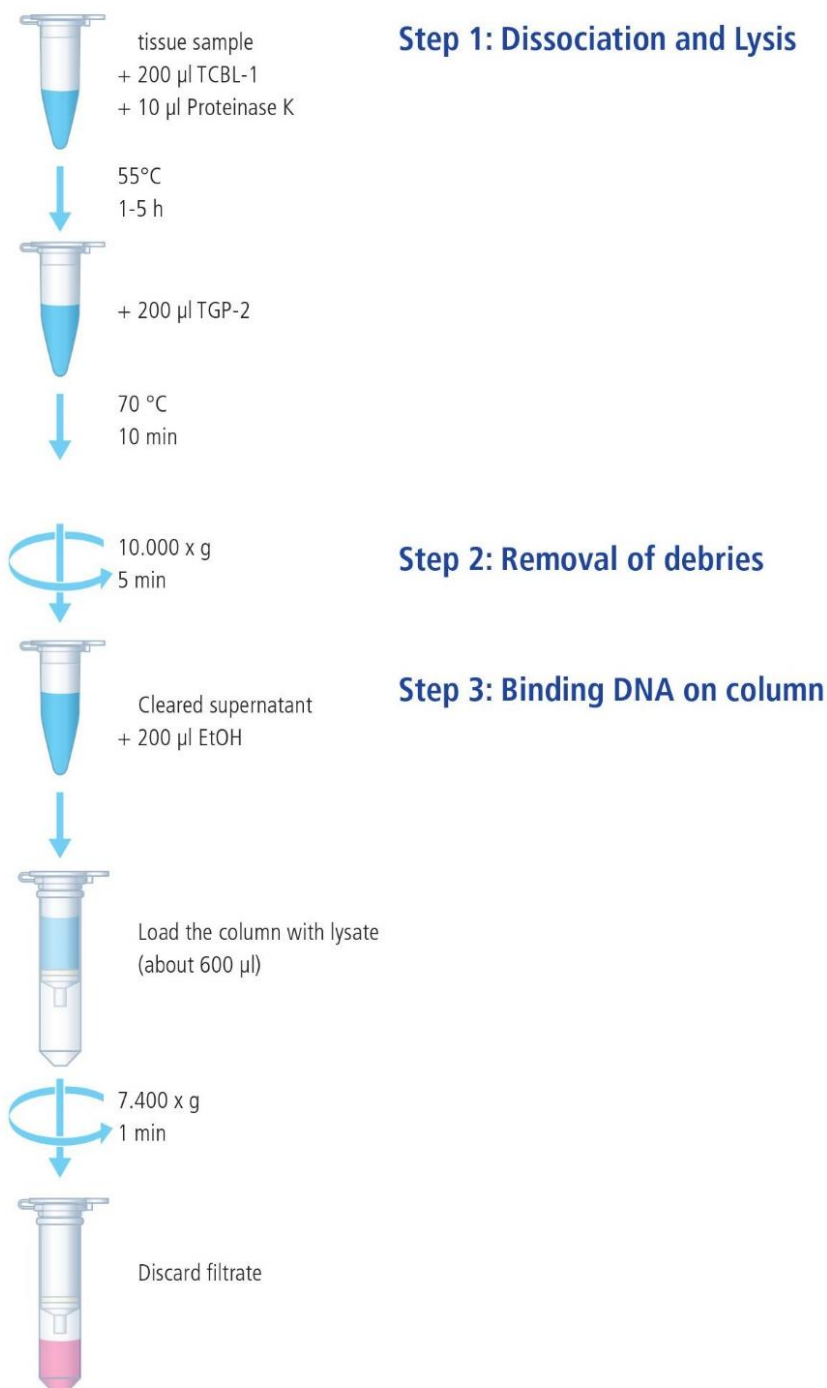
6.4 Extraction Workflow

1. Set up incubator/ heat shaker/ water bath to 55 °C.
2. Add **200 µl Lysis Buffer TCBL-1 + 10 µl Proteinase K Solution** to the sample. Vortex thoroughly at maximum speed for 1 minute.
3. Incubate the tube at 55 °C until complete digestion of tissue in heat shaker (1 hour up to overnight).
4. Add **200 µl Binding Buffer TGP-2** to lysate and vortex for 10 seconds.
5. Incubate the solution at 70°C for 10 minutes.
6. Centrifuge the tube at 13000 rpm (approx. 10000 g) for 5 minutes and transfer the DNA-containing supernatant in a new tube (removal of debris by centrifugation).
7. Add **200 µl ethanol** to DNA solution and vortex for 10 seconds.
8. Place a spin column in a provided 2 ml collection tube.
9. Apply the **600 µl liquid sample** to the column and centrifuge the tube at 10000 rpm (approx. 7400 g) for 1 minute.
10. Discard flow through. Place the column back into the same tube. Collection tubes are re-used to reduce plastic waste.
11. Add **500 µl Wash Buffer TGI-3** (add isopropanol before use, as indicated on the bottle) to the column and centrifuge at 10000 rpm (approx. 7400 g) for 1 minute.
12. Discard flow through. Place the column back into the same tube.
13. Add **500 µl Wash Buffer TE-4** (add ethanol before use as indicated on the bottle) to the column and centrifuge at 10000 rpm (approx. 7400 g) for 2 minutes to remove complete wash buffer.
14. Place the column in a 1.5 ml receiver tube (not provided) and **heat the column at 70°C for 2 - 5 minutes** to remove residues of alcohol. Add **50 - 100 µl Elution Buffer EL-3** (heated up to 55 - 70°C) to the column and incubate for 2 minutes at room temperature.
15. Centrifuge at 10000 rpm (approx. 7400 g) for 1 minute and collect the eluate and proceed with down-stream processing (gel electrophoresis, PCR etc.)

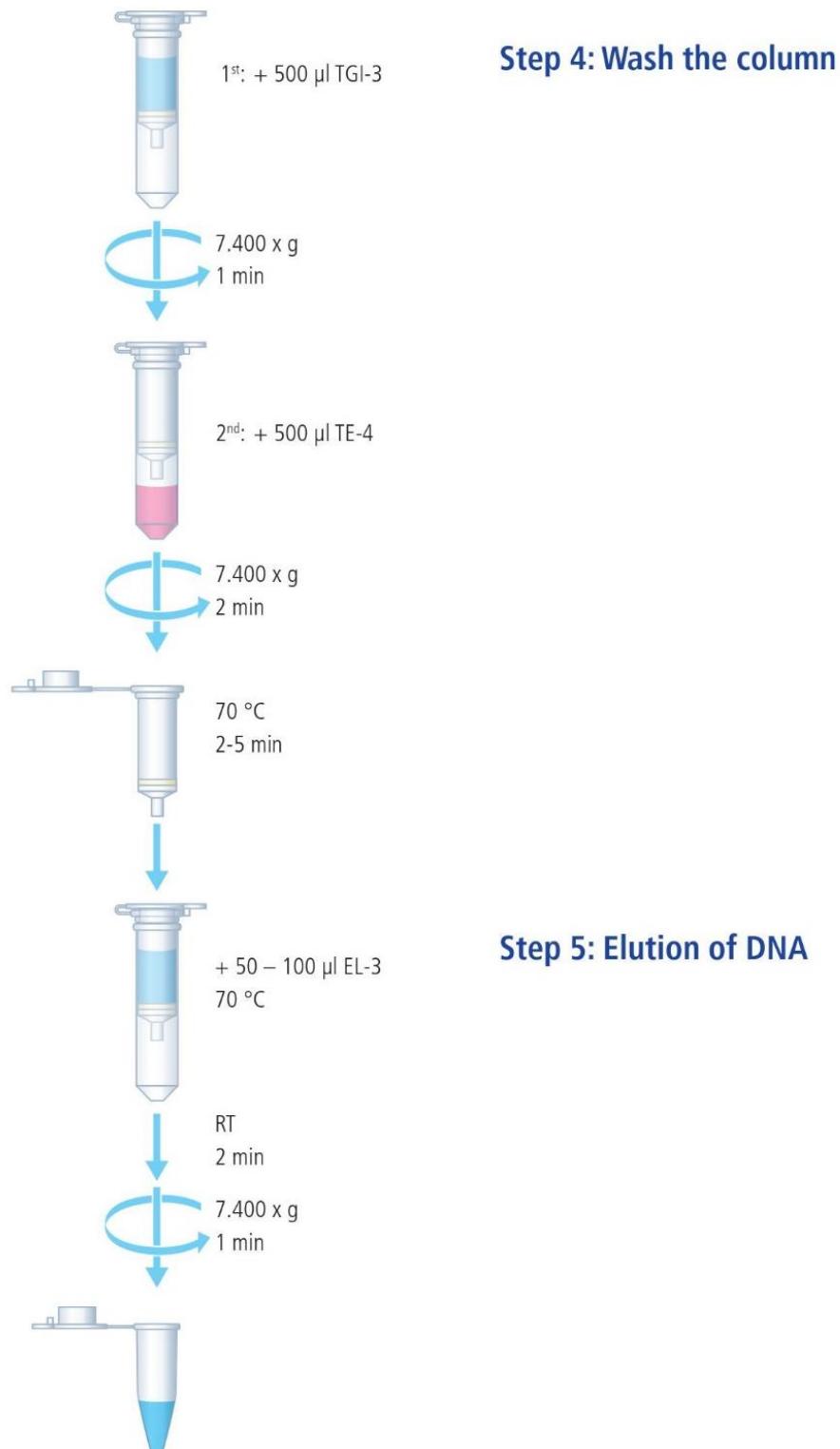
Note: it is very important to remove residues of alcohol and wash buffer since it can decrease DNA recovery.

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7. Flowchart of Extraction



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8. Troubleshooting

This guide can help solve problems that may arise. BIORON GmbH welcomes comments and suggestions for improvement and supplement of our protocols or any hints on other molecular biology applications. The BIORON team is always pleased to answer any of your questions about our products.

Observation	Possible cause	Comments/suggestions
Low or no DNA yield	Inefficient lysis of sample Lysis Buffer TCBL-1 not added Binding Buffer TGP-2 not added	Proteinase K added to Lysis Buffer? Stored at -20°C? Ensure that Lysis Buffer TCBL-1 has been added and mixed with the lysate. Ensure that Binding Buffer TGP-2 has been added to and mixed with the lysate
DNA "smear"	Nuclease activity/ contamination	Upon disintegration of samples, cellular nucleases are released and may degrade genomic DNA. Whenever possible, fresh samples should be used and processed immediately. Use only sterilized glass and plastic ware in order to avoid nuclease contamination.
Low DNA performance	Salt in eluate	Make sure that you followed all washing steps of the procedure.

Technical Support

If you have questions or suggestions for improvement of this kit, please send us an email: info@bioron.net

9. Warranty and Guarantee of Products

The manufacturer guarantees the performance of its Ron's Tissue DNA Mini Kit in the manner described in this handbook. It is up to the user to determine the suitability of Ron's Tissue DNA Mini Kit for its particular use. In case a product fails to perform due to any reason except misuse, the manufacturer will replace it without further charge or refund the purchase price. We reserve the right to change, alter or modify our Ron's Tissue DNA Mini Kit to enhance its performance and design. The manufacturer's terms and conditions are available on request.

10. Limitations of Product Use

The use of all products of **Ron's Purification Kits** is strictly limited to general lab usage. They are not to be applied for any diagnostic use, including human or drug purposes.



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Space for your Notes

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