BIORON life science

Ron's Cell-free DNA Extraction Kit

Kit for the isolation of cell-free / circulating tumor DNA from human plasma and serum

Research Use Only (RUO)

Content

Ron's Cell-free DNA Extraction Kit Sample volume: 500 µl – 1 ml	Ref. No: 804050 (50 preps)
Ron's Cell-free DNA Extraction Kit Sample volume: > 1 ml – 4 ml	Ref. No: 804060 (50 preps)
Valid from:	December 2020

Re03122020

Ron's Cell-free DNA Extraction Kit

1. Introduction

The Ron's Cell-free DNA Extraction Kit is designed for preparation of cell-free DNA from plasma and serum.
Important: The kit is not suited for DNA extraction of blood or other cell containing samples.

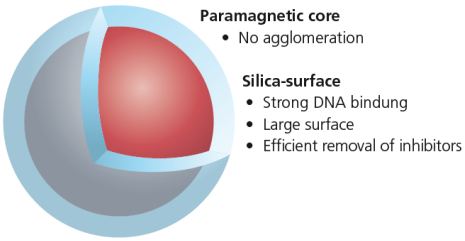
In Ron's Cell-free DNA Extraction Kit neither chaotropic salts nor strong ionic detergents are used.

The kit contains magnetic beads and buffers for lysis, DNA binding, washing and elution of DNA into small volume. Each kit contains a manual with detailed protocols of DNA extraction.

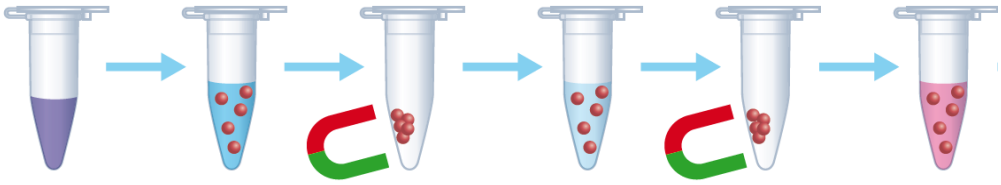
The Ron's Cell-free DNA Extraction Kit presents remarkable features of timesaving, easy, prompt and high yield DNA purification.

The procedure is based on optimized buffers and the use of our specially designed **Ron's Magnetic Beads**. The advanced buffer system is optimized for efficient recovery of DNA and removal of contaminants. DNA is adsorbed by the unique Ron's Magnetic Beads, all impurities are efficiently removed by washing steps. The cell-free DNA is directly eluted in a special buffer.

Novel magnetic beads with a special silica surface



DNA extraction: graphical flow chart



Lysis & binding	Binding of DNA on nano silica magnetic beads	Separation of beads with strong magnets	Wash beads two times to remove inhibitors	Separation of beads with strong magnets	Elution under strong conditions	Analyse with qPCR or dPCR

2. Content of the Kit

Ref No	804050 50 preps Sample volume: 500 µl – 1 ml	804060 50 preps Sample volume: 1 ml – 4 ml	Storage
Ron's cf-Magnetic Beads-Si	250 µl	350 µl	2-8°C
Collection tubes 2.0 ml *	50	50	RT
Lysis & Binding Buffer cfLB-Si-1	2 x 50 ml	2 x 100 ml	2-8°C
Wash Buffer cfWB-Si-1	1 x 10 ml	1 x 10 ml	2-8°C
Wash Buffer cfWB-Si-2	1 x 25 ml	1 x 25 ml	2-8°C
Elution Buffer cfEL-Si-1	3 x 1,8 ml	3 x 1,8 ml	2-8°C
Manual	1	1	-

*Low DNA binding tubes

3. Storage Conditions and Stability

Magnetic beads of the kit are filled in tubes and show full performance in this condition at 2 - 8 °C for 1 year. Store buffers at 2 - 8 °C.
Guarantee for full performance of the kit as specified in this manual is only valid if the storage conditions are complied.

4. Quality Control

The setting up of the **Ron's Cell-free DNA Extraction Kit** is monitored routinely on a lot-to-lot basis.

5. Safety Information

The following components of **Ron's Cell-free DNA Extraction 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 sheets, which can be requested from the manufacturer. There is no need of labeling harmful features with H & P phrases upon packing sizes of 125 ml or 125 g.

Component	Hazard content	GHS symbol		Hazard phrases	Precaution phrases
Lysis & Binding Buffer cfLB-Si-1	Detergent Tween 20 0.1- 1%		Warning	302, 319	280, 301+312 305+351+338, 330, 337+313
Wash Buffer cfWB-Si-1	Detergent Tween 20 0.1- 1%		Warning	302, 319	280, 301+312 305+351+338, 330, 337+313
Elution Buffer cfEL-Si-1	CABS pH 11.5		Warning	302, 319	280, 301+312 305+351+338, 330, 337+313

Hazard phrases	
H302	Harmful if swallowed
H315	Cases skin irritation
H319	Causes serious eye irritation
H334	May cause allergy asthma symptoms or breathing difficulties if inhaled
H335	May cause respiratory irritation

Precaution phrases	
P280	Wear protective gloves / eye protection
P301+312	If swallowed: call a poison center/doctor/ …/ if you well unwell
P302+352	If on skin: wash with plenty of water
P304+340	If inhaled: go to fresh air and keep at rest in a position comfortable for breathing
P305+351+338	If in eyes: rinse cautiously with clean water for several minutes. Remove contact lenses. Continue rinsing
P312	Call a poison center/doctor/../ if you feel unwell
P330	Rinse mouth
P332+313	If skin irritation occurs: get medical advice/ attention
P337+313	If eye irritation persists: get medical advice/attention
P342+311	If experiencing respiratory symptoms: call a poison center/ doctor
P403+233	Store in a well ventilated place. Keep container tightly closed

6. Protocol

Additional material required :

- Incubator/ heat shaker or water bath
- Receiver tubes (1.5 ml, 2 ml), low DNA binding
- 15 ml or 50 ml tubes
- Magnetic rack (for 2 ml cups, see our Magnetic Rack for 12x 2 ml) BIORON´s MagicMag Rack Ref.No BI87000)

Note: Preparation of plasma or serum

Multiple of 2 ml plasma volume

- The protocol is optimised for 2 ml plasma. For larger volume please use multiple of 2 ml and prepare separately.

Ratio Lysis& Binding Buffer to plasma volume

- Lysis & Binding Buffer cfLB-1 is a **2x concentrate** and must be added to plasma volume in an equimolar ratio (e.g. 2 ml plasma + 2 ml Lysis & Binding Buffer cfLB-1 with a final volume of 4 ml etc.)

Use of magnetic rack

For separation of 2 ml plasma (or multiples) magnetic rack are used (see our MagicMag Rack).

Use of magnetic beads

Resuspension of the magnetic particles is critical to achieve optimal results. Resuspend magnetic particles carefully by mixing or vortexing before use.

6. Protocol with work flow for plasma and serum with magnetic rack

Pretreatment: Work flow for 500 µl - 1 ml and > 1- 4 ml plasma, serum: please select

First work flow: Transfer **0,5 ml** up to **1 ml** plasma in tube (2 ml or 15 ml):

- Add **500 µl (or 1 ml)** Lysis & DNA Binding Buffer cfLB-Si-1 **to 500 µl (or 1 ml)** plasma/serum (equimolar ratio) and go to step 1

Second work flow: Transfer **2 ml** or multiple of **2 ml** (separately) plasma/serum into 15 ml or 50 ml tube

- Add **2 ml** Lysis & DNA Binding Buffer cfLB-Si-1 to 2 ml plasma/serum and go to step 1

Step 1:Lysis

Mix for 30 seconds resulted solutions and incubate for 20 min at room temperature (RT) and continue with DNA binding

Step 2: DNA binding

Important: mix beads by vortexing before adding to the sample

Add **5 µl** Ron's cf-Magnetic Beads-Si to the **1 ml** mixture or **7 µl Beads** to the mixture from **1ml to 2ml.**

- Mix gently and incubate 20 min at room temperature (pipet up & down or use rotating mixer with low speed).
- After binding: for higher volume > 2 ml, settle down the beads to bottom of tube and discard supernatant with a rest of 2 ml (including magnetic beads). Resuspend beads in a 2 ml tube and transfer tube (with beads and plasma solution) to the magnetic rack.

Step 3: Wash procedure

- Separate beads with magnetic rack by folding up the magnetic bar and discard supernatant
- **1st wash: add 200 µl Wash Buffer cfWB-Si-1**
- Resuspend beads by pipetting up&down
- Separate beads with magnetic rack and discard supernatant
- **2nd wash: add 500 µl Wash Buffer cfWB-Si-2**
- Resuspend beads by pipetting up&down
- Separate beads with magnetic rack and discard supernatant
- **3rd wash: add 500 µl Wash Buffer cfWB-Si-2**
- Resuspend beads by pipetting up&down
- Separate beads with magnetic rack and discard supernatant

Step 4: Elution

- Add to the collected beads 30 -100 µl Elution buffer and mix
- Incubate beads with Elution buffer for **5 min at 80**°, mix several times
- Collect beads with Magnetic rack
- **Transfer supernatant with DNA!!** into a new cup and analyze 1 - 5 µl eluate with qPCR

- **Note:** It is infeasible to analyse the extracted DNA by gel-electrophoreses or photospectrometry, because the resulted product is single-stranded DNA

7. 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	Lysis & Binding Buffer cfLB-1 and or magnetic beads not added Wash Buffer cfWB-1 not added	Ensure that Lysis & Binding Buffer cfLB-1 has been added and mixed with the plasma or serum. Ensure that Washing Buffer cfWB-1 has been added for washing
Low DNA performance	Salt in eluate	Make sure that you followed all washing steps of the procedure and elution.

8. Warranty and Guarantee of Products

The manufacturer guarantees the performance of its Ron's Cell-free DNA Extraction Kit in the manner described in this handbook. 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 Cell-free DNA Extraction Kit to enhance its performance and design. The manufacturer’s terms and conditions are available on request.

9. **Limitations of Product Use**

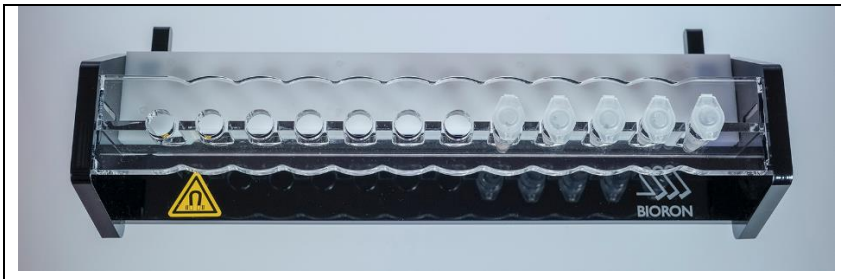
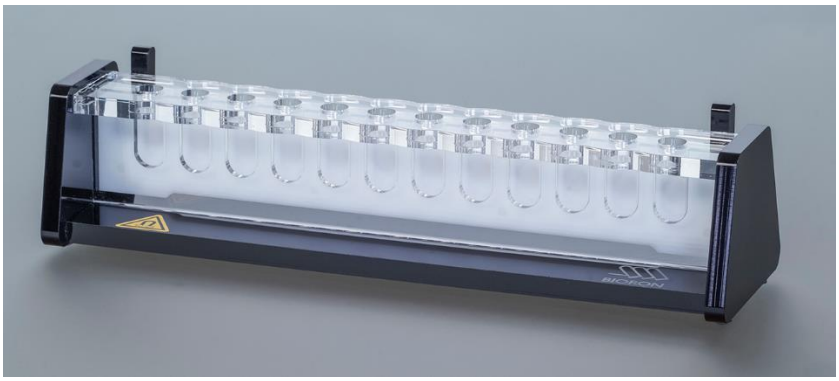
The use of all products of **Ron’s Purification Kits** is strictly limited to research purposes. They are not to be applied for any diagnostic use, including human or drug purposes.

10. MagicMag Rack

MagicMag Rack for separation of suspension with magnetic beads

- BIORON´s Magnetic Rack has a tilt mechanism!
- There is no need to move the tubes during the purification process;
- it is the magnet itself which will be turned towards the tubes or away for the washing and incubation steps.

The rack can be used with 12 tubes (1.5 ml or 2.0 ml) simultaneously and is equipped with strong magnets for a short incubation time.

	**Magic Mag Rack** Supervision with hinged magnetic strip
	Front view with folded magnetic strip

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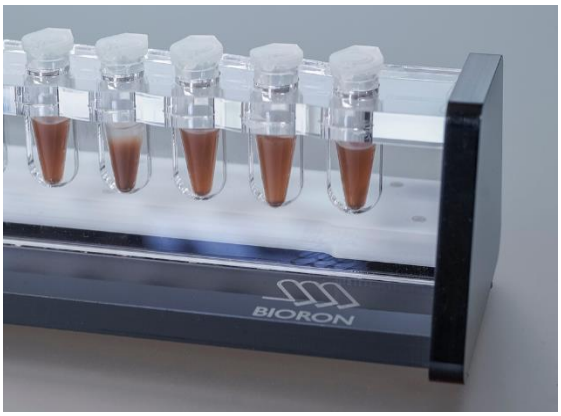	**Magic MagRack** Suspension with magnetic beads With hinged magnetic strip
	Separated magnetic beads (without supernatant) with folded magnetic strip

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