Blood RNA Extraction Kit MANUAL

GENEDIA™ **life Science Co**.

Product # EK0850R

 # EK08100R

 # EK08200R

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Introduction

The **GENEDIA™ Blood RNA Extraction Kit** (spin column) is a fast, simple, and inexpensive means of preparing genomic RNA for amplification. In order to extract RNA from blood, genomic RNA can be extracted from whole blood.

The kit is even suitable to use with whole blood treated with either citrate or EDTA.

Kit Specifications

Purification is based on spin column chromatography using **GENEDIA**'s proprietary resin as the separation matrix. The RNA is preferentially purified from other cellular components without the use of phenol or chloroform. First, samples are digested with the provided Proteinase K and Digestion Buffer -BRL- using an incubation time which is specific for the recovery of RNA. Next, Absolute Ethanol is then added to the lysate, and the solution is loaded onto a spin-column. GENEDIA's column binds nucleic acids in a manner that depends on ionic concentrations, thus only the RNA will bind to the column while the contaminants will be removed in the flowthrough or retained on the top of the resin. The bound RNA is then washed with the provided Wash Solution in order to remove any impurities, and the purified RNA is eluted with BRE Buffer.
BRA (RBC Lysis Buffer) is designed to lyse **red blood cells** without affecting leukocytes, normal tissue, or tumor cells.

Kit Components

Components	Product # EK0850R (50 preps)	Product # EK08100R (100 preps)	Product # EK08200R (200 preps)
BRA (RBC Lysis)	45 ml	90 ml	90 ml*2
BRL (Lysis)	10ml	20 ml	40ml
BRWB1 (Wash 1)	12 ml	22 ml	43 ml
BRWB2 (Wash 2)	7 ml	13 ml	25 ml
BRE (Elution)	2 ml	4 ml	8 ml
Proteinase K	0.5 ml	1 ml	1 ml*2
Spin column	50	100	200
Product insert	1	1	1

Storage Conditions

All components of the **GENEDIA™ Blood RNA Extraction Kit** should be stored at room temperature (20-25 °C) and are stable for 1 year. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

Recommended Equipment and Reagents

- 56-65°C incubator
- Sampler in 100 to 1000 Microliter size
- Sampler tips

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- Benchtop microcentrifuge (Not provided)
- Micropipettors
- 2 ml microcentrifuge tubes
- Vortex
- Absolute Ethanol
- DNase (if DNA-free RNA is required)

Precautions and Disclaimers

- Prior to using the protocol, Genetic ID recommends that care be taken in homogenizing the sample in a manner that minimizes the risk of inadvertently contaminating the sample. Contamination can occur using improperly cleaned equipment or using poor laboratory practices during homogenization, weighing and labeling of the subsample.
- The Buffer BRL contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.
- All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

Where RCF = required gravitational acceleration (relative centrifugal force in units of g);
r = radius of the rotor in cm; and RPM = the number of revolutions per minute required to achieve the necessary g-force.

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.

Notes Prior to Use

• Prepare a working concentration of the **BRWB1** by adding:

- **5 ml** of 96 - 100% ethanol (not provided) to each of the bottles containing **12 ml** of concentrated **BRWB1**. This will give a final volume of 17 ml for **Product # EK0850R**
- **9 ml** of 96 - 100% ethanol (not provided) to the supplied bottle containing **22 ml** concentrated **BRWB1**. This will give a final volume of 31 ml for **Product # EK08100R**
- **18 ml** of 96 - 100% ethanol (not provided) to the supplied bottle containing **43 ml** concentrated **BRWB1**. This will give a final volume of 61 ml for **Product # EK08200R**

The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

• Prepare a working concentration of the **BRWB2** by adding:

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- **10 ml** of 96 - 100% ethanol (not provided) to each of the bottles containing **7 ml** of concentrated **BRWB2**. This will give a final volume of 17 ml for **Product # EK0850R**
- **19 ml** of 96 - 100% ethanol (not provided) to the supplied bottle containing **13 ml** concentrated **BRWB2**. This will give a final volume of 32 ml for **Product # EK08100R**
- **37 ml** of 96 - 100% ethanol (not provided) to the supplied bottle containing **25 ml** concentrated **BRWB2**. This will give a final volume of 62 ml for **Product # EK08200R**

The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

Sampling and Extraction Procedure

A. Sample preparation

1) Add **250µl** of whole blood in 2 ml microcentrifuge tubes (Not provided).
2) Transfer **300µl** of **BRA** on microcentrifuge tubes (Not provided) and shake until the precipitate gets mixed well.
3) Centrifuge at **3,000 x g** (~4,000 RPM) for **3 minutes**.
4) Discard supernatant through reversal and save the pellet; while removing the supernatant, be careful to not discard the gelatinous appearing pellet.
5) Repeat steps 2 to 4 by **300 µl** of **BRA** for 2 times.
6) Add **200 µl of BRL** & **10µl Proteinase K** of solution (20mg/ml) to the sample and vortex for few minutes and incubates at **58°C for 10 minutes**. After the lysing process wait for the sample to reach room temperature.

(Optional): *DNase Treatment: If DNA-free RNA is required, add the equivalent of 100 units of DNase to the lysate.*

7) Proceed to binding step (step 8).

B. Binding RNA to Column

8) Add **400 µl** Absolute Ethanol (not provided) and mix by vortexing (2 x 5 s).
9) Assemble a Spin column with collection tube.
10) Transfer lysate onto the column and incubate for **2 minutes at room temperature**.
11) Centrifuge at **8,000 x g** (~6,000 RPM) for **2 minutes**.
12) Discard the flowthrough. Reassemble the spin column with its collection tube

Typically, samples will pass through the columns within ≤ 1 minute (in less than 1 minute). If the entire volume has not passed, spin for an additional minute

C. Column Wash

1st wash

13) Add **300 µl Buffer BRWB1** to the GENEDIA RNA Spin column.
14) Centrifuge at **11,000 x g** (~10,000 RPM) for **1 minutes**.
15) Discard the flowthrough. Reassemble the spin column with its collection tube.

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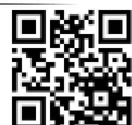

2nd wash

16) Add **300 µl Buffer BRWB2** to the column and centrifuge for **1 minutes at 11,000 x g** (~10,000 RPM).
17) Discard the flowthrough and reassemble the spin column with its collection tube.
18) Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

D. Elute RNA

19) Place the Spin column in a microcentrifuge tube (provided).
20) Add **40 µl of BRE** (preheated to 60°C) to the column.
21) Incubate at **room temperature** for **2 minutes**.
22) Centrifuge at **12,000 x g** (~12,000 rpm) for **1 minutes**.

<u>Note</u>: *For an improved yield, elute the sample twice and use after concentration process*

E. Storage of RNA

The purified RNA may be stored at –20°C for a few weeks. It is recommended that samples be placed at –70°C for long term storage.

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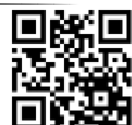

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
No RNA purified	**Reagents added incorrectly**	Add buffers in the correct order so that the sample is bound, washed and eluted in the correct sequence.
	Reagents added incorrectly	Be sure that buffers have been reconstituted correctly, and that reagents have been added in the correct order.
	Insufficient number of blood cells in the starting sample	Increase the volume of the starting sample (up to 1000 µl).
	Incomplete elution during preparation	Larger elution volumes and longer incubation times can sometimes increase the amount purified RNA.
Low RNA performance	**Ethanol has been carried over**	Centrifuge 2nd wash for 1 minute to ensure complete removal.
The spin column is clogged.	**Inefficient cell lysis**	Check Protease K activity. Also ensure that correct volume of Lysis Buffer was added to the blood sample
	Cell debris may be clogging the column	When a high cell number is expected in the blood sample, ensure that the optional spin for 2 minutes at 14,000 rpm after the Proteinase K incubation is performed. Take the clean supernatant only for the binding step
	The sample is too large	Too many cells were applied to the column. Ensure that Proteinase K and Lysis Buffer are proportionally added as the blood volume is increased. Clogging can be alleviated by centrifuging for a longer period of time until the lysate passes through the column.
Small amounts or no nucleic acids in the elute	**Proteinase K digestion**	For certain sample types and for RNA isolation use of Proteinase K is required for the sample lysis step. Compare protocols with and without Proteinase K digestion.
	Nucleic acids degraded	Samples should be processed immediately. If necessary, add RNase inhibitor to the sample. Create a nuclease-free environment and ensure that no nucleases are present. Use suitable tips and buffer reservoirs. Check that all buffers have been prepared and stored correctly.

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Isolation Method of RNA from Blood

Workflow

RBC Lysis
250µl of blood + 300µl of BRA & incubate for 5 min at 4 °C
Centrifuge at 3,000xg 3 min
Repeat by adding 300µl of BRA for 2 times.

Sample Lysis
Add 200 µl of BRL & 10µl of Proteinase K to cell pellet & vortex & incubate for 10 min at 58 °C

Nucleic acid Binding
Add 400µl Ethanol
Transfer onto the column & Incubate for 2 min at RT

Flow Through
Centrifuge at 8,000xg 2 min
Discard the flowthrough

1st Wash
Add 300 µl BRWB1 and Centrifuge at 11,000xg 1 min
Discard the flowthrough

2nd Wash
Add 300 µl BRWB2 and Centrifuge at 11,000xg 1 min
Discard the flowthrough

Elution
Place the column into an Elution tube Add 40 µl BRE & Incubate for 2min at RT & Centrifuge 12,000xg 1 min

Purified RNA

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