Blood DNA Extraction Kit MANUAL

GENEDIA™ **life Science Co**.

Product #EK0550R

#EK05100R

#EK05200R

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Introduction

The **GENEDIA™ Blood DNA Extraction Kit** provides a simple, nontoxic method for efficiently isolating high-molecular-weight DNA from tissue, whole blood. DNA isolated with the DNA Extraction Kit is free from contaminants and may be used directly for restriction digests, cloning, Southern blotting, PCR amplification, and other DNA analysis techniques. **GENEDIA™ Blood DNA Extraction Kit** is a modification of a procedure based on separating contaminating protein from DNA by salt precipitation.the procedure involves digestion of cellular proteins, subsequent removal of the proteins by "salting out" using standard sodium chloride, precipitation of the DNA with ethanol and resuspension in the buffer of choice. The number of samples that may be processed simultaneously using this technique is limited only by the centrifuge space available.

Kit Specifications

The **GENEDIA™ Blood DNA Extraction Kit** is based on a four-step process. The first step in the purification procedure lyses the cells and the nuclei. For isolation of DNA from white blood cells, this step involves lysis of the red blood cells in the BBA Solution, followed by lysis of the white blood cells and their nuclei in the BLB2 Solution. An RNase digestion step may be included at this time; it is optional for some applications. The cellular proteins are then removed by a salt precipitation step, which precipitates the proteins but leaves the high molecular weight genomic DNA in solution. Finally, the genomic DNA is concentrated and desalted by alcoholic precipitation. DNA purified with this system is suitable for a variety of applications, including amplification, digestion with restriction endonucleases and membrane hybridizations (e.g., Southern and dot/slot blots).

Kit Components

Components	Product # EK0550R (50 preps)	Product # EK05100R (100 preps)	Product # EK05200R (200 preps)
BBA	110 ml	110 ml*2	110 ml*4
BLB2	15 ml	30 ml	60 ml
GBC	4 ml	8 ml	16 ml
GBD	15 ml	30 ml	60 ml
GE	10 ml	20 ml	40 ml
Elution tubes (1.7 mL)	50	100	200
Product Insert	1	1	1

Storage Conditions

All components of the **GENEDIA company® Blood Extraction kit** should be stored at room temperature (20-25 °C) and are stable for 1 year under the mentioned condition.

Recommended Equipment and Reagents

- 60-65°C incubator
- Sampler in 100 to 1000 Microliter size
- Sampler tips
- Benchtop microcentrifuge

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- Micropipettors
- 2 mL microcentrifuge tubes
- Vortex

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.
- 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.

Sampling and Extraction Procedure

A. Sample preparation

1) Add **500µL** of total EDTA peripheral blood in 2 ml microcentrifuge tube (Not provide).
2) Transfer **700 µl** of **BBA** buffer on microcentrifuge tube and shake until the precipitate gets mixed well.
3) Centrifuge at **3000 rpm** at **24° C** 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 **BBA** for 2 times.

B. Lysate Preparation

6) Add **300 µl** of **BLB2** buffer to it and strike the bottom of the microcentrifuge tube until a foamy texture appears and the pellet disappears.

Note: *If precipitation occurs in the BLB2 (usually due to cold temperature) then the buffer must be warmed up to 30 - 40 °C and thoroughly mixed in order to completely solubilize its contents.*

7) Incubate for **10 minutes** at **room temperature**.
8) At intervals of **30-40 seconds**, hit the bottom of the microcentrifuge tube.
9) Add **80 µl** of **GBC** and reverse the tube several times slowly.
10) Add **300 µl** of **GBD** and shake it until it becomes milky.
11) Centrifuge the microcentrifuge tube at **11,000 x g** (~ 10,000 RPM) for **10 minutes**.

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12) Three phases will be formed in microcentrifuge tube

C. DNA precipitation

13) Pick **300 µl** of the top aqueous phase carefully, avoid picking up the middle layer.
14) Transfer aqueous phase to a new microcentrifuge tube (provided).
15) Add **600 µl** of **absolute ethanol** and reverse the microcentrifuge tube a few times. A cloudy type DNA precipitation must be seen. Then centrifuge for **1 minute** at **11,000 x g** (~ 10,000 RPM).
16) Discharge the supernatant.

D. Washing step

17) Add **600 µl** of **70% ethanol** and centrifuge it for **1 minute** at **11,000 x g** (~ 10,000 RPM).
18) Discharge the supernatant

E. Elute DNA

19) The supernatant must be completely discarded; Air dry for **10 minutes**
20) Re-suspend the pellet in **200 µl GE buffer.**
21) Incubate for **10 to 20 minutes** at **65 °C** to homogenize extracted DNA.

F. Storage of DNA

The purified DNA 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 DNA purified	**Reagents added incorrectly**	Add buffers in the correct order so that the sample is bound, washed and eluted in the correct sequence.
Low DNA yield	**Incomplete lysis**	Pellet must be completely resuspended before addition of Lysis Buffer (BLB2) – color should change from light to dark pink.
	Incomplete elution	Larger elution volumes and longer incubation times can increase yield.
	Reagents added incorrectly	Be sure that buffers have been reconstituted correctly, and that reagents have been added in the correct order.
	Incomplete elution during preparation	Larger elution volumes and longer incubation times can sometimes increase yield. For elution of DNA >10 kb, heat the DNA Elution Buffer to 50°C and extend incubation time to 5 minutes. Multiple rounds of elution can also be performed.
Low DNA performance	**Ethanol has been carried over**	Centrifuge final wash for 1 minute to ensure complete removal.
	Excessive salt in sample	Use 70% ethanol and do not skip wash steps.

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