Spin Micro DNA Extraction Kit

REF 2-020

Σ 20 Extractions

18-25°C

RUO

For research use only

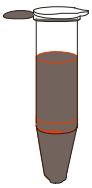
1.	**Lysis Buffer** *(brown cap)*	2x 2 ml	Warning
2.	**Binding Buffer** *(red cap)*	3x 2 ml	
3.	**Protease** *(orange cap)*	lyophilized	Danger
	Add 250 µl sterile distilled water and mix well.		
	Store dissolved Protease at -20°C.		
4.	**Wash Buffer 1**	15 ml	Warning
	Add 15 ml 99-100% ethanol and mix well.		
5.	**Wash Buffer 2**	9 ml	
	Add 21 ml 99-100% ethanol and mix well.		
6.	**Elution Buffer** *(violet cap)*	3x 2 ml	
7.	**Spin Filter**	20	
8.	**Receiver Tubes 2.0 ml**	40	
9.	**Receiver Tubes 1.5 ml**	20	
10.	**Instructions For Use**	1	

ViennaLab Diagnostics GmbH
Gaudenzdorfer Guertel 43-45
A-1120 Vienna, Austria
Phone: (+43-1) 8120156-0
info@viennalab.com

ViennaLab®

www.viennalab.com

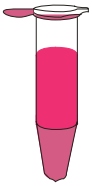
SCHEME



Transfer the sample into a 1.5 ml reaction tube.

Add **50 µl Lysis Buffer** (buccal swabs: use 200 µl Lysis Buffer) and **10 µl Protease**. Close tube and vortex for 5 sec.

Incubate for **5-20 min.** (according to starting material) at **56°C**.

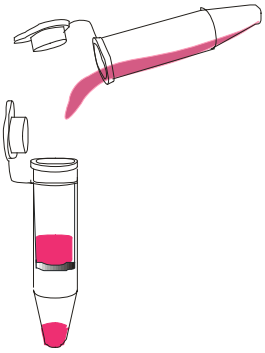


Add **100 µl Binding Buffer** (buccal swabs: use 400 µl Binding Buffer). Mix thoroughly with a pipette.

Place a fresh **Spin Filter** into a **Receiver Tube 2.0 ml.**

Transfer the lysate onto the **Spin Filter.**

Centrifuge for **1 min.** at **12,000-14,000 rpm**.



Add **300 µl Wash Buffer 1**.

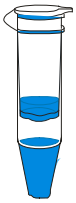
Centrifuge for **30 sec.** at **12,000-14,000 rpm**.

Place the **Spin Filter** into a new **Receiver Tube 2.0 ml**.

Add **750 µl Wash Buffer 2**.

Centrifuge for **30 sec.** at **12,000-14,000 rpm**. Discard filtrate.

Place the **Spin Filter** again into the tube and centrifuge for **2 min.** at **12,000-14,000 rpm**.

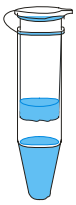


Place the **Spin Filter** into a **Receiver Tube 1.5 ml**.

Add **200 µl prewarmed (56°C) Elution Buffer**.

Incubate for **1 min.** at **room temperature**.

Centrifuge for **1 min.** at **8,000 rpm**.



Discard **Spin Filter**.

genomic DNA

Instructions for use

I. INTENDED USE

Kit for isolation and purification of genomic DNA from human whole blood samples and human buccal swabsn. *For research use only. Not for human in vitro diagnostics.*

II. METHODOLOGY

The procedure includes four steps: (1) lysis of cells, (2) DNA binding to the membrane of a Spin Filter, (3) washing of the membrane and elimination of ethanol, (4) elution of DNA.

Starting Material	Yield	Time	Purity
• 50 µl whole blood • buccal swab	up to 2 µg DNA depending on type and amount of starting material	20 - 40 min.	typical A_{260nm} : A_{280nm} ratio: 1.7 - 2.0

III. KIT COMPONENTS

See list of all kit components on page I.

Warning: Lysis Buffer contains ammonium chloride
(H302, H315, H319; P280, P305 + P351 + P338)

Danger: Protease
(H315, H319, H335; P280, P305 + P351 + P338)

Warning: Wash Buffer 1 contains guanidine thiocyanate
(H302, H312, H332, EUH 032; P273)

Store all reagents at room temperature (18-25°C).
Store Protease dissolved in sterile distilled water at -20°C !

IV. MATERIALS REQUIRED BUT NOT SUPPLIED

In addition to standard molecular biology laboratory equipment, the following is needed:

- Adjustable microcentrifuge capable of 8,000-14,000 rpm (6,000-16,000 x g)
- Thermoblock or thermomixer capable of 56°C (± 2°C)
- Vortex mixer
- 99-100% ethanol
- Sterile distilled water

V. ASSAY PROCEDURE

1. DNA Isolation from Whole Blood

Use fresh or frozen blood with EDTA or citrate anticoagulant; avoid blood containing heparin. Do not store blood for more than 3 days at ambient temperature or more than 1 week at 2-8°C before use. Blood which has been kept frozen for more than one year, or gone through more than three freeze-thaw cycles is unsuitable to be used in this procedure.
If cryoprecipitates (formed during thawing of frozen samples) are visible, avoid aspirating them as they could clog the Spin Filter membrane.

*Prewarm **Elution Buffer** to **56°C** in the thermoblock.*
*Before first use of the kit, add 250 µl **sterile distilled water** to Protease and 15 ml **99-100% ethanol** to Wash Buffer 1 and 21 ml **99-100% ethanol** to Wash Buffer 2.*

- Transfer **50 µl blood sample** into a 1.5 ml reaction tube.
- Add **50 µl Lysis Buffer** and **10 µl Protease**. Close tube and vortex for 5 sec.
- Incubate for **5 min.** at **56°C** in the thermoblock.
- Add **100 µl Binding Buffer** and mix thoroughly with a pipette.
- Place a fresh **Spin Filter** into a **Receiver Tube 2.0 ml.**
- Transfer the lysate onto the **Spin Filter**. Close the Spin Filter with the tube cap.
- Centrifuge for **1 min.** at **12,000-14,000 rpm** (12,000-16,000 x g) in a microcentrifuge.
- Open the cap and add **300 µl Wash Buffer 1**. Close the Spin Filter.
- Centrifuge for **30 sec.** at **12,000-14,000 rpm** in a microcentrifuge.
- Transfer the Spin Filter into a new **Receiver Tube 2.0 ml**.
- Add **750 µl Wash Buffer 2**. Close the Spin Filter.
- Centrifuge for **30 sec.** at **12,000-14,000 rpm** in a microcentrifuge.
- Discard the filtrate and place the Spin Filter again into the same Receiver Tube 2.0 ml.
- Centrifuge for **2 min.** at **12,000-14,000 rpm** in a microcentrifuge.
  Pay attention to completely remove ethanol-containing Wash Buffers!
- Transfer the Spin Filter into a new **Receiver Tube 1.5 ml**.
- Add **200 µl** of **prewarmed (56°C) Elution Buffer**.
- Incubate for **1 min.** at **room temperature**.
- Centrifuge for **1 min.** at **8,000 rpm** (6,000 x g) in a microcentrifuge.
- Discard the Spin Filter.

The resulting filtrate contains genomic DNA suitable for various downstream applications (e.g. PCR, restriction enzyme digestion, cloning, sequencing, Southern blotting).
DNA should be kept refrigerated (2-8°C; up to one week) or frozen at -20°C.

2. DNA Isolation from Buccal Swabs

*Prewarm **Elution Buffer** to **56°C** in the thermoblock.*
*Before first use of the kit, add 250 µl **sterile distilled water** to Protease and 15 ml **99-100% ethanol** to Wash Buffer 1 and 21 ml **99-100% ethanol** to Wash Buffer 2.*

- Add **200 µl sterile distilled water**, **200 µl Lysis Buffer** and **10 µl Protease** to a 2 ml tube.
- Cut and insert the sampling zone of the **buccal swab** into the tube. Close the vial.
- Incubate for **20 min.** at **56°C** in the thermoblock.
- Open the cap, pick the swab with clean tweezers, squeeze it against the wall and remove it from the tube.
- Add **400 µl Binding Buffer** and mix thoroughly with a pipette.
- Transfer the lysate into a fresh **Spin Filter** on top of a **Receiver Tube 2.0 ml**, and proceed with the protocol as described for whole blood *(chapter V/1)*.

VI. QUALITY CONSIDERATIONS

- A thorough understanding of the procedure outlined here, and precise laboratory equipment and techniques are required to obtain reliable results.
- Do not use Spin Micro DNA Extraction Kit components beyond the expiration date printed on the outside of the kit box. Do not mix reagents from different lots.
- Avoid microbial contamination and cross-contamination of reagents or samples by using sterile disposable pipette tips throughout. Do not interchange bottle caps.

VII. SAFETY

- Do not drink, eat, smoke, or apply cosmetics in designated work areas. Wear laboratory coats and disposable gloves when handling specimens and kit reagents. Wash hands thoroughly afterwards.
- Handle specimens as if capable of transmitting infectious agents. Thoroughly clean and disinfect all materials and surfaces that have been in contact with specimens. Discard all waste associated with clinical specimens in a biohazard waste container.
- Avoid contact of Lysis Buffer, Binding Buffer, Protease and Wash Buffer 1 with skin, eyes, or mucous membranes. If contact does occur, immediately wash with large amounts of water. If spilled, dilute with water before wiping dry.
- Adhere to all local and federal safety and environmental regulations which may apply.

H302: Harmful if swallowed
H312: Harmful in contact with skin
H315: Causes skin irritation
H319: Causes serious eye irritation
H332: Harmful if inhaled
H335: May cause respiratory irritation
P273: Avoid release to the environment
P280: Wear protective gloves/protective clothing/eye protection/face protection
P305+351+338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
EUH 032: Contact with acids liberates very toxic gas

VIII. TROUBLESHOOTING

Advise on troubleshooting may be obtained by contacting ViennaLab through the local distributor or directly at techhelp@viennalab.com.

Problem	Possible Cause	Comments/Suggestions
Low amount of DNA	• Insufficient lysis	• Continuous shaking is crucial for improving lysis efficiency • Increase lysis time • Reduce amount of starting material
	• Inefficient binding of DNA to Spin Filter membrane	• Sample must be thoroughly mixed with Binding Buffer (pipetting or vortexing) prior to transfer into the Spin Filter • Check correct amount of Binding Buffer
	• Incomplete elution	• Check addition of correct amounts of ethanol to both Wash Buffers • Increase centrifugation time for complete removal of ethanol • Increase incubation time with prewarmed Elution Buffer to 2-5 min. • Prewarm Elution Buffer to 80°C • Increase Elution Buffer volume
	• Low concentration of extracted DNA	• Elute DNA with lower volume of Elution Buffer
Degraded or sheared DNA	• Old or incorrectly stored starting material	• Ensure the samples are collected and stored as described • Avoid repeated freezing-thawing of the material • Old material may contain degraded DNA
Problems in subsequent applications (e.g. PCR)	• Ethanol carryover in the eluate	• Increase centrifugation time for complete removal of ethanol
	• Salt carryover in the eluate	• If salt precipitates have formed in Wash Buffers during storage, dissolve them by moderate warming

REF		Σ
2-014	GEN^{X}TRACT™ Blood DNA Extraction System	100 extractions
2-020	Spin Micro DNA Extraction Kit	20 extractions
2-030	D2PCR™ Buffer	100 extractions

Distributed by:

Manufacturer:

ViennaLab Diagnostics GmbH
Gaudenzdorfer Guertel 43-45
A-1120 Vienna, Austria
Phone: (+43-1) 8120156-0
info@viennalab.com

ViennaLab®

www.viennalab.com