

Spin Micro DNA Extraction Kit



2-020



20 extractions



18-25°C

1. Lysis Buffer (<i>brown cap</i>)	2x 2 ml		R 36
2. Binding Buffer (<i>red cap</i>)	3x 2 ml		R 11/36/67
3. Protease (<i>orange cap</i>)	lyophilized		R 36/37/38
<i>Add 250 µl sterile distilled water and mix well.</i>			
<i>Store dissolved Protease at -20°C.</i>			
4. Wash Buffer 1	15 ml		R 20/21/22/32
<i>Add 15 ml 99-100% ethanol and mix well.</i>			
5. Wash Buffer 2	9 ml		
<i>Add 21 ml 99-100% ethanol and mix well.</i>			
6. Elution Buffer (<i>violet cap</i>)	3x 2 ml		
7. Spin Filter	20		
8. Receiver Tubes 2.0 ml	40		
9. Receiver Tubes 1.5 ml	20		

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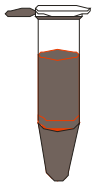
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SCHEME

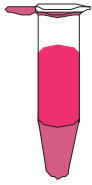


Transfer the sample into a 1.5 ml reaction tube.

Add **100 µl Lysis Buffer** (blood samples and cell suspensions: use 50 µl Lysis Buffer only) and **10 µl Protease**. Close tube and vortex for 10 sec.



Incubate for **5-30 min.** (according to starting material) at **56°C** under continuous shaking.



Add **100 µl Binding Buffer** (dried blood spots: use 200 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.**

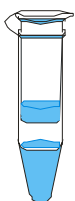
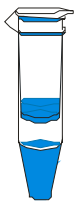
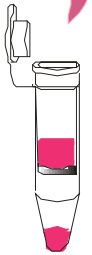
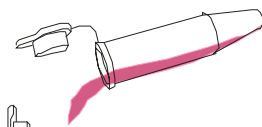
Place the **Spin Filter** into a **Receiver Tube 1.5 ml.**

Add **200 µl prewarmed (56°C) Elution Buffer** (dried blood spots: use 50-100 µl Elution Buffer only).

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, dried blood spots (filter paper punches), small tissue samples, body fluids and eukaryotic cells.

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
<ul style="list-style-type: none"> • 50 µl whole blood • dried blood spots (2-4 mm ø) • 10^2 - 10^5 eukaryotic cells • body fluids (10^2 - 10^5 cells) • 0.5 - 5 mg tissue 	<p>up to 2 µg DNA</p> <p>depending on type and amount of starting material</p>	20 - 50 min.	<p>typical</p> <p>$A_{260} : A_{280}$ ratio:</p> <p>1.7 - 2.0</p>

III. KIT COMPONENTS

See list of all kit components on page I.

- Lysis Buffer contains ammonium chloride (R 36).
- Binding Buffer contains 2-propanol (R 11/36/67).
- Protease (R 36/37/38).
- Wash Buffer 1 contains guanidine thiocyanate (R 20/21/22/32).

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)
- Thermomixer capable of 56°C (\pm 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 thermomixer.

Before first use of the kit, add **sterile distilled water** to Protease and **99-100% ethanol** to Wash Buffer 1 and 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 thermomixer under continuous shaking.
- 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 into 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 Dried Blood Spots

Use blood spotted and dried on filter paper (e.g. 903[®] specimen collection paper), Guthrie test cards or similar blood collection cards.

Store blood spots on filter paper or collection cards dry at 2-8°C.

Cut disk(s) of 2-4 mm diameter from the blood spot using a suitable micropunch.

Prewarm **Elution Buffer** to **56°C** in the thermomixer.

Before first use of the kit, add **sterile distilled water** to Protease and **99-100% ethanol** to Wash Buffer 1 and Wash Buffer 2.

- Transfer a **blood card disk** into a 1.5 ml reaction tube.
Using up to 3 disks from the same blood spot can increase DNA yields.
- Add **120 µl sterile distilled water**. Close tube and vortex for 10 sec.
- Incubate for **10 min.** at **room temperature**.
- Add **100 µl Lysis Buffer** and **10 µl Protease**. Close tube and vortex for 10 sec.
- Incubate for **10 min.** at **56°C** in the thermomixer under continuous shaking.

- Add **200 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*).

To increase DNA concentration, use smaller volumes of Elution Buffer (50-100 µl) !

3. DNA Isolation from Eukaryotic Cell Pellets (10^2 - 10^5 Cells)

Use fresh cells or cells that have been stored frozen at or below -20°C.

*Prewarm **Elution Buffer** to **56°C** in the thermomixer.*

*Before first use of the kit, add **sterile distilled water** to Protease and **99-100% ethanol** to Wash Buffer 1 and Wash Buffer 2.*

- Pellet **cells** by centrifugation in a 1.5 ml reaction tube. Remove the supernatant.
- Add **100 µl Lysis Buffer** and **10 µl Protease**. Close tube and vortex for 10 sec.
- Incubate for **10 min.** at **56°C** in the thermomixer under continuous shaking.
- Add **100 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*).

4. DNA Isolation from Cell Suspensions and Body Fluids (10^2 - 10^5 Cells in 50 µl)

Use fresh cell suspension or body fluid (e.g. amniotic fluid, synovial fluid), or material that has been stored frozen at or below -20°C.

*Prewarm **Elution Buffer** to **56°C** in the thermomixer.*

*Before first use of the kit, add **sterile distilled water** to Protease and **99-100% ethanol** to Wash Buffer 1 and Wash Buffer 2.*

- Transfer **50 µl cell suspension** or **body fluid** 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 thermomixer under continuous shaking.
- Add **100 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*).

5. DNA Isolation from Small Tissue Samples (0.5 - 5 mg)

Use fresh or frozen tissue samples (e.g. biopsy, frozen section) of max. 5 mg.

*Prewarm **Elution Buffer** to **56°C** in the thermomixer.*

*Before first use of the kit, add **sterile distilled water** to Protease and **99-100% ethanol** to Wash Buffer 1 and Wash Buffer 2.*

- Transfer **tissue sample** into a 1.5 ml reaction tube.
Mechanical grinding (e.g. with a pipette tip) will support lysis.
- Add **100 µl Lysis Buffer** and **10 µl Protease**. Close tube and vortex for 10 sec.
- Incubate for **30 min.** at **56°C** in the thermomixer under continuous shaking.
If necessary, lysis time may be further increased.
- Centrifuge for **2 min.** at **maximum speed** in a microcentrifuge.
- Transfer the **supernatant** into a fresh 1.5 ml reaction tube.
- Add **100 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.

VIII. TROUBLESHOOTING

Advice on troubleshooting may be obtained from the summary on page III, as well as by contacting ViennaLab through the local distributor or directly at the address provided on page I.

Problem	Possible Cause	Comments/Suggestions
Low amount of DNA	<ul style="list-style-type: none"> • Insufficient lysis • Inefficient binding of DNA to Spin Filter membrane • Incomplete elution • Low concentration of extracted DNA 	<ul style="list-style-type: none"> • Continuous shaking is crucial for improving lysis efficiency • Increase lysis time • Reduce amount of starting material • Sample must be thoroughly mixed with Binding Buffer (pipetting or vortexing) prior to transfer into the Spin Filter • Check correct amount of Binding Buffer • 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 • Elute DNA with lower volume of Elution Buffer
Degraded or sheared DNA	<ul style="list-style-type: none"> • Old or incorrectly stored starting material 	<ul style="list-style-type: none"> • 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)	<ul style="list-style-type: none"> • Ethanol carryover in the eluate • Salt carryover in the eluate 	<ul style="list-style-type: none"> • Increase centrifugation time for complete removal of ethanol • If salt precipitates have formed in Wash Buffers during storage, dissolve them by moderate warming

REF

2-014
2-020

GEN^XTRACT Blood DNA Extraction System
Spin Micro DNA Extraction Kit



100 extractions
20 extractions

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