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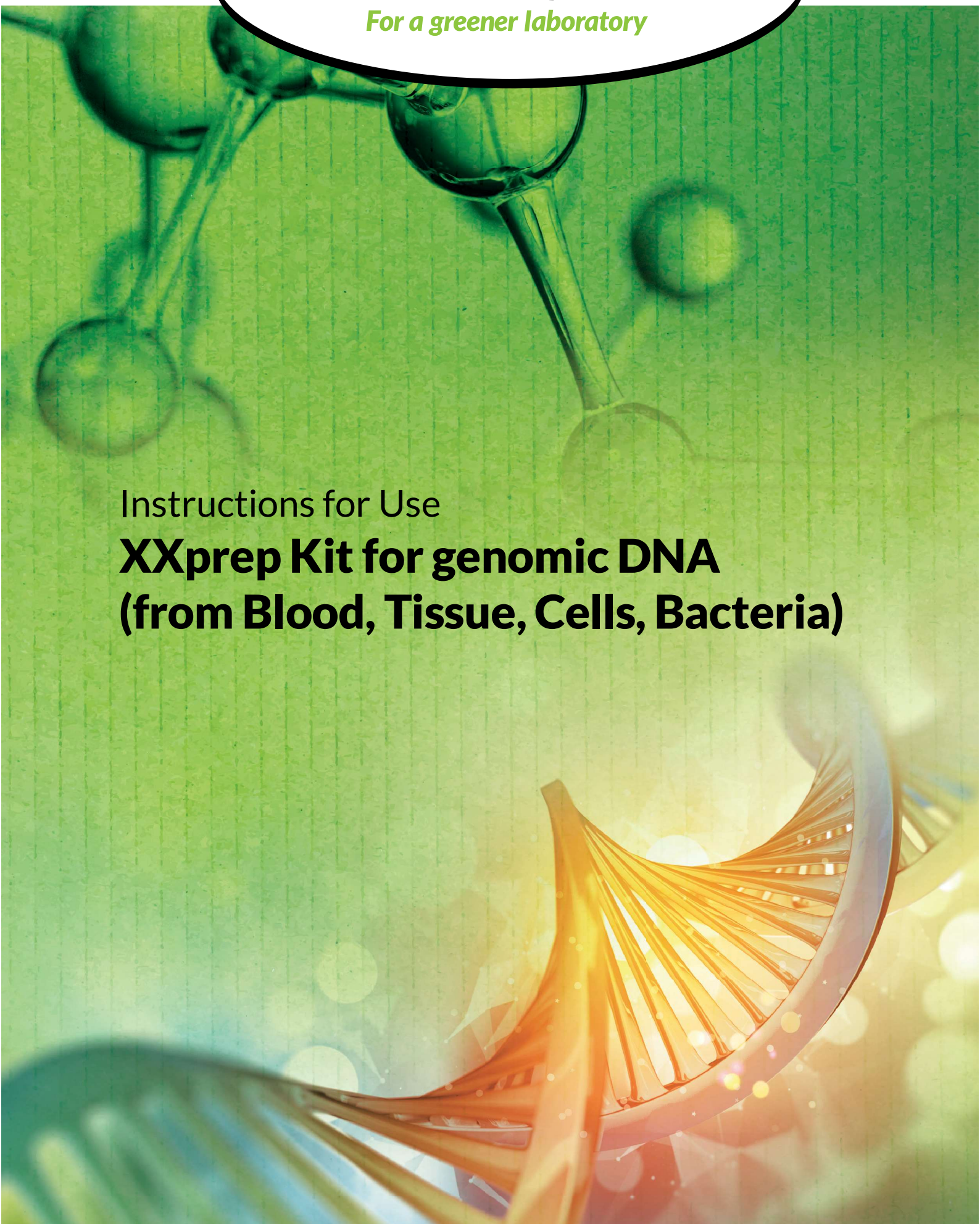

Instructions for Use

XXprep Kit for genomic DNA (from Blood, Tissue, Cells, Bacteria)

Published July 2021, TDS_EN_2710_XXprep-Kit-for-genomic-DNA
This Instruction for Use describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!
The latest version is available via the following QR code:

Order no.

2710RC010	XXprep Kit for genomic DNA (from Blood, Tissue, Cells, Bacteria), 10 Reactions
2710RC050	XXprep Kit for genomic DNA (from Blood, Tissue, Cells, Bacteria), 50 Reactions
2710RC250	XXprep Kit for genomic DNA (from Blood, Tissue, Cells, Bacteria), 250 Reactions

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1 Introduction

1.1 Intended use

The XXprep Kit for genomic DNA has been designed as a very efficient tool for fast isolation of genomic DNA from a wide range of starting materials like whole blood (fresh or frozen blood; stabilized with EDTA or citrate, from common blood collection systems), tissue, rodent tails, eucaryotic cells as well as bacteria.

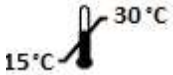
The extraction procedure is based on a new patented chemistry and combines sample lysis with subsequent binding of nucleic acids onto the surface of a Spin Filter membrane. After several washing steps the nucleic acids are eluted from the membrane by using elution buffer. Extraction chemistry and extraction protocol are optimized to get maximum of yield.

The kit is intended for use by professional users. The kit has been designed to be used for a wide range of different downstream applications, like amplification reactions and further analytical procedures.

CONSULT INSTRUCTION FOR USE
This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual

For easy reference and orientation, the manual uses the following warning and information symbols as well as the shown methodology:

Symbol	Information
	REF Catalogue number.
	Content Contains sufficient reagents for <N> reactions.
	Storage conditions Store at room temperature or shown conditions respectively.
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
	Lot number The number of the kit batch.
	Manufactured by Contact information of manufacturer.
	For single use only Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the kit and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → “Notes on the use of this manual" p. 3).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully prior to guarantee your own safety and a trouble-free operation.
Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

FOR SINGLE USE ONLY!
This kit is made for single use only!

ATTENTION!
Don't eat or drink components of the kit!
The kit shall only be handled by educated personal in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles to avoid any injuries. This kit could be used with potential infectious samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Please observe the federal, state and local safety and environmental regulations. Follow the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

ATTENTION!
Do not add bleach or acidic components to the waste after sample preparation!

NOTE
Emergency medical information in English and German can be obtained 24 hours a day from:
Poison Information Center, Freiburg / Germany
Phone: +49 (0)761 19 240.

For more information, please ask for the material safety data sheet (MSDS).

3 Storage conditions

Store lyophilized Proteinase K and the dissolved Proteinase K at 4 °C to 8 °C.

All other components of the XXprep Kit for genomic DNA should be stored dry at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions dissolve these precipitates by careful warming.

4 Functional testing and technical assistance

The neoFroxx GmbH guarantees the correct function of the kit for applications as described in the manual. This kit was produced and tested in an ISO 13485 certified facility.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the XXprep Kit for genomic DNA or other products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 (0) 6251 989240. For other countries please contact your local distributor.

5 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Product specifications” p. 8). Since the performance characteristics of neoFroxx GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of neoFroxx GmbH kits using other protocols than those described below. neoFroxx GmbH kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA ´88 regulations in the U.S. or equivalent regulations required in other countries.

All products sold by neoFroxx GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
The kit is for research use only!

6 Kit components

6.1 Included kit components

	Σ 10	Σ 50	Σ 250
REF	2710RC010	2710RC050	2710RC250
Lysis Solution CBV	5 ml	25 ml	120 ml
Binding Solution BL	8 ml	40 ml	200 ml
Binding Solution SBS	2 ml	12 ml	60 ml
Proteinase K	for 2 x 0.3 ml working solution	for 2 x 1.5 ml working solution	for 6 x 1.5 ml working solution
Washing Solution C	5 ml	25 ml	120 ml
Washing Solution BS (conc.)	2 ml	8 ml	2 x 18 ml
Washing Solution MS (conc.)	6 ml	30 ml	2 x 66 ml
Spin Filter	10	50	5 x 50
Receiver Tubes	50	5 x 50	25 x 50
Manual	1	1	1

6.2 Components not included in the kit

- 1.5 ml and 2.0 ml tubes
- 96–99.8 % ethanol (molecular biology grade, undenaturated)
- ddH_2O for dissolving Proteinase K
- RNase free water or 10 mM Tris- HCl pH 8.0 – 8.5 for elution steps
- 1 x PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)
- RNase A (10 mg/ml); optional

7 Product specifications

1. Starting material:
   - 200 / 400µl fresh or frozen whole blood samples (stabilizers: EDTA or citrate)
   - Cell cultures (max. 5 x 10^6 cells)
   - Tissue samples (max. 20 mg)
   - Rodent tails (0.2 – 0.8 cm tail)
   - Bacterial cell cultures (max. 10^{10} cells)

NOTE
Avoid freezing and thawing of starting material.

2. Time for isolation:
   - Approximately 10 minutes after each lysis step
3. Typical yield:
   - Up to > 100 µg gDNA
   - Typical ratio A_{260}:A_{280}: 1.8–2.0
   - Typical ratio A_{260}:A_{230}: 1.8–2.3

8 Initial steps before starting

- Heat thermal mixer or water bath at 60 °C or 56°C depends on used protocol
- Add to Proteinase K the indicated amount of ddH_2O, mix thoroughly and store as described above.

2710RC010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
2710RC050 2710RC250	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add to Washing Solution BS (conc.) the indicated amount of absolute ethanol, mix thoroughly and store as described above.

2710RC010	Add 18 ml ethanol to 2 ml Washing Solution BS (conc.).
2710RC050	Add 72 ml ethanol to 8 ml Washing Solution BS (conc.).
2710RC250	Add 162 ml ethanol to 18 ml Washing Solution BS (conc.).

- Add to Washing Solution MS (conc.) the indicated amount of absolute ethanol, mix thoroughly and store as described above.

2710RC010	Add 14 ml ethanol to 6 ml Washing Solution MS (conc.).
2710RC050	Add 70 ml ethanol to 30 ml Washing Solution MS (conc.).
2710RC250	Add 154 ml ethanol to 66 ml Washing Solution MS (conc.).

- Centrifugation steps should be carried out at room temperature.
- For Lysis steps we recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3–4 times during the incubation. No shaking will reduce the lysis efficiency.
- Pre-fill the needed amount of RNase free water or 10 mM Tris-HCl into a 2.0 ml reaction tube and incubate at 60 °C until the elution step.

9 Protocols for DNA isolation from blood samples

9.1 Protocol 1: Isolation from 200 µl blood samples

IMPORTANT
If the sample volume is less than 200 µl, add the appropriate volume of PBS.

1. Pipette 200 µl of whole blood sample into a 1.5 ml reaction tube.
2. Add 200 µl Lysis Solution CBV and 20 µl Proteinase K, mix vigorously by pulsed vortexing for 10 seconds and incubate the sample at 60 °C for 10 minutes.

NOTE
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of Binding Solution BL, vortex shortly and incubate for 5 minutes at room temperature.

3. Optional: centrifuge the 1.5 ml reaction tube for 10 seconds to remove condensate from the lid of the reaction tube.
4. Open the 1.5 ml reaction tube and add 350 µl Binding Solution BL to the lysed sample. Mix carefully by pipetting up and down several times (3 – 4 times), apply the sample using the pipette to a Spin Filter located in a Receiver Tube and close the cap of the Spin Filter.

IMPORTANT NOTE
Don't vortex the sample at this step!
It is important that the sample and the Binding Solution BL are mixed by pipetting up and down several times. Vortexing will lead to reduced yield of DNA.

5. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time

6. Open the Spin Filter and add 400 µl Washing Solution C, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

7. Open the Spin Filter and add 600 µl Washing Solution BS, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter into a new Receiver Tube.

8. Open the Spin Filter and add 600 µl Washing Solution BS, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

9. Centrifuge at max. speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.

10. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add 200 µl RNase free water or 10 mM Tris HCl (pH 8 – 8.5) (pre-warmed at 60 °C). Incubate at room temperature for 2 minutes.

11. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Two elution steps with equal volumes of pre-warmed RNase free water or 10 mM Tris HCl (pH 8 – 8.5) (e.g. 100 µl + 100 µl) might increase the yield of extracted gDNA.

NOTE
The DNA can be eluted with a lower or a higher volume of µl RNase free water or 10 mM Tris HCl (pH 8 – 8.5) (depends on the expected yield of genomic DNA). Elution with lower volumes increases the final concentration of DNA. Store the extracted DNA at 4 °C to 8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

9.2 Protocol 2: Isolation from 400 µl blood samples

IMPORTANT
If the sample volume is less than 400 µl, add the appropriate volume of PBS.

1. Pipette 400 µl of whole blood sample into a 2.0 ml reaction tube.
2. Add 400 µl Lysis Solution CBV and 30 µl Proteinase K, mix vigorously by pulsed vortexing for 10 seconds and incubate the sample at 60 °C for 10 minutes.

NOTE
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of Binding Solution BL, vortex shortly and incubate for 5 minutes at room temperature.

3. Optional: centrifuge the 1.5 ml reaction tube for 10 seconds to remove condensate from the lid of the reaction tube.
4. Open the 1.5 ml reaction tube and add 700 µl Binding Solution BL to the lysed sample. Mix carefully by pipetting up and down several times (3 – 4 times), apply 750 µl of the sample using the pipette to a Spin Filter located in a Receiver Tube and close the cap of the Spin Filter.

IMPORTANT NOTE
Don't vortex the sample at this step!
It is important that the sample and the Binding Solution BL are mixed by pipetting up and down several times. Vortexing will lead to reduced yield of DNA.

5. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

6. Apply the residual sample to the Spin Filter. Close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

7. Open the Spin Filter and add 400 µl Washing Solution C, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

8. Open the Spin Filter and add 600 µl Washing Solution BS, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter into a new Receiver Tube.

9. Open the Spin Filter and add 600 µl Washing Solution BS, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

10. Centrifuge at max. speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.

11. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add 200 µl RNase free water or 10 mM Tris HCl (pH 8 – 8.5) (pre-warmed at 60 °C). Incubate at room temperature for 2 minutes.

12. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Two elution steps with equal volumes of pre-warmed RNase free water or 10 mM Tris HCl (pH 8 – 8.5) (e.g. 100 µl + 100 µl) might increase the yield of extracted gDNA.

NOTE
The DNA can be eluted with a lower or a higher volume of RNase free water or 10 mM Tris HCl (pH 8 – 8.5) (depends on the expected yield of genomic DNA). Elution with lower volumes increases the final concentration of DNA. Store the extracted DNA at 4 °C to 8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

10 Protocol for DNA isolation from tissue samples or rodent tails

1. Cut max. 20 mg of tissue sample or rodent tails into small pieces and place the tissue in a 1.5 ml or 2.0 ml reaction tube. Add 400 µl Lysis Solution CBV and 25 µl Proteinase K.

2. Mix vigorously by pulsed vortexing for 5 s. Incubate at 56°C until the sample is completely lysed (appr. 0.5 – 3 h for tissue sample and appr. 2 h for rodent tails).

IMPORTANT
The lysis step should be finished if the material is completely lysed. Optional centrifuge the 1.5 ml reaction tube at maximum speed for 3 minutes to spin down unlysed material. Transfer the supernatant carefully into another 1.5 ml tube.

NOTE
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of Binding Solution BL, vortex shortly and incubate for 5 minutes at room temperature.

3. Add 200 µl Binding Solution SBS to the lysed sample, mix the sample by pipetting up and down several times. It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

4. Apply the sample to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (12.000 rpm) for 2 minutes.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

5. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

6. Open the Spin Filter and add 650 µl Washing Solution MS, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the filtrate. Place the Spin Filter back into the Receiver Tube.

7. Open the Spin Filter and add 650 µl Washing Solution MS, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the filtrate. Place the Spin Filter back into the Receiver Tube.

8. Open the Spin Filter and add 300 µl Washing Solution MS, close the cap and centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube and place the Spin Filter into a 1.5 ml tube.

9. Carefully open the cap of the Spin Filter and add 100–400 µl RNase free water or 10 mM Tris HCl (pH 8 – 8.5). Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. A second elution step will increase the yield of extracted DNA.

NOTE
The DNA can be eluted with a lower or a higher volume of water or 10 mM Tris- HCl (depends on the expected yield DNA). Elution with lower volumes of Elution Buffer increases the final concentration of DNA. Store the extracted DNA at 4 °C to 8 °C. For long time storage placing at -18 °C to -22 °C is recommended.

11 Protocol for DNA Isolation from cell cultures

1. Pellet cells (max. 5 x 10^6 cells) by centrifugation for 10 min at 5.000 x g (7.500 rpm). Discard supernatant.
2. Add 100 µl 1 x PBS Buffer and resuspend the cell pellet completely by pipetting up and down several times.
3. Add 300 µl Lysis Solution CBV and 25 µl Proteinase K and mix vigorously by pulsed vortexing for 5 s. Incubate at 56°C until the sample is completely lysed (appr. 15 – 30 minutes depends on number of cells).

NOTE
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of Binding Solution BL, vortex shortly and incubate for 5 minutes at room temperature.

4. Add 200 µl Binding Solution SBS to the lysed sample, mix by vortexing or by pipetting up and down several times. It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.
5. Apply the sample to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (12.000 rpm) for 2 minutes.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

6. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
7. Open the Spin Filter and add 650 µl Washing Solution MS, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the filtrate. Place the Spin Filter back into the Receiver Tube.

8. Open the Spin Filter and add 650 µl Washing Solution MS, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the filtrate. Place the Spin Filter back into the Receiver Tube.

9. Open the Spin Filter and add 300 µl Washing Solution MS, close the cap and centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube and place the Spin Filter into a 1.5 ml tube.

10. Carefully open the cap of the Spin Filter and add 100–400 µl RNase free water or 10 mM Tris HCl (pH 8 – 8.5). Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. A second elution step will increase the yield of extracted DNA.

NOTE
The DNA can be eluted with a lower or a higher volume of RNase free water or 10 mM Tris HCl (pH 8 – 8.5) (depends on the expected yield DNA). Elution with lower volumes increases the final concentration of DNA. Store the extracted DNA at 4 °C to 8 °C. For long time storage placing at -18 °C to -22 °C is recommended.

12 Protocol for DNA Isolation from bacterial cell cultures

12.1 Collection of bacterial cells

1. Transfer the bacterial culture (volume depends on the concentration of starting material) into a tube 2.0 ml or 1.5 ml tube depends on initial volume.
2. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 minutes at 3,000 x g). Discard the supernatant. Do not discard the pellet!
3. Resuspend the bacterial cell pellet in 100 µl TE-Buffer.

12.2 Enzymatic lysis

12.2.1 Gram-negative bacteria

NOTE
Although Gram-negative bacteria do not require a pre-lysis step, using Lysozyme (not included in the kit) can enhance the efficiency of lysis.

1. Add 20 µl Lysozyme (10mg/ml, 400 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
2. Proceed with “Proteolytic lysis step” on p.19.

12.2.2 Gram-positive bacteria

NOTE
Gram-positive bacteria require a pre-lysis step using Mutanolysin and/or Lysozyme (not included in the kit).

1. Add 20 µl Lysozyme (10mg/ml, 400 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
2. Add 5 µl Mutanolysin (0.4 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.

NOTE
Lysozyme and Mutanolysin exert synergistic activity. Using both enzymes together will increase the yield of isolated nucleic acids.

3. Proceed with “Proteolytic lysis step” on p.19.

12.2.3 Staphylococcus strains

NOTE
For pre-lysis of *Staphylococcus* the enzyme Lysostaphin is recommended (not included in the kit).

1. Add 10 µl Lysostaphin (0.4 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
2. Proceed with "Proteolytic lysis step" on p.19.

12.3 Proteolytic lysis

1. Add 280 µl Lysis Solution CBV and 20 µl Proteinase K to the sample and vortex the sample shortly.
2. Incubate the sample for 30 minutes at 60 °C and 550 rpm in a thermoshaker.
3. Continue with "Bacterial DNA extraction" on p. 15.

NOTE
The kit co-purifies DNA and RNA, if DNA and RNA in the sample. If RNA-free genomic DNA is required, add 2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of Binding Solution SBS, vortex shortly and incubate for 10 minutes at room temperature

12.4 Bacterial DNA extraction

1. Add 200 µl Binding Solution SBS to the lysed sample, mix by pipetting up and down several times.

IMPORTANT
It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

2. Apply the sample to the Spin Filter located in a Receiver Tube. Close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 2 minutes.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

3. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

4. Open the Spin Filter and add 650 µl Washing Solution MS, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the filtrate. Place the Spin Filter back into the Receiver Tube.

5. Open the Spin Filter and add 650 µl Washing Solution MS, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the filtrate. Place the Spin Filter back into the Receiver Tube.

6. Open the Spin Filter and add 300 µl Washing Solution MS, close the cap and centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube and place the Spin Filter into a 1.5 ml tube.

7. Carefully open the cap of the Spin Filter and add 50–200 µl RNase free water or 10 mM Tris HCl (pH 8 – 8.5). Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. A second elution step will increase the yield of extracted DNA.

NOTE

The DNA can be eluted with a lower or a higher volume of RNase free water or 10 mM Tris- HCl (depends on the expected yield of bacterial DNA). Elution with lower volumes increases the final concentration of DNA. Store the extracted DNA at 4 °C to 8 °C. For long time storage placing at -18 °C to -22 °C is recommended.

13 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient disruption or homogenization	Increase lysis time. Increase centrifugation speed. Reduce amount of starting material.
Little or no DNA eluted	
Insufficient lysis	Increase lysis time. Reduce amount of starting material. Overloading of Spin Filter reduces yield!
Incomplete elution	Prolong the incubation time with Elution Buffer to 5 minutes or repeat elution step once again. Take a higher volume of Elution Buffer.
Insufficient mixing with Binding Solution BL or Binding solution SBS	Mix sample with Binding Solution BL or Binding solution SBS by pipetting up and down several times prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted DNA	
Too much RNase free H_2O or 10 mM Tris HCl (pH 8 – 8.5)	Elute the DNA with lower volume of Elution Buffer.
Degraded or sheared DNA	
Incorrect storage of starting material	Ensure that the starting material is frozen immediately in liquid N_2 or in minimum at 20° C and is stored continuously at -80° C! Avoid thawing of the material.
Old material insufficient	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	
RNA contaminations of extracted DNA	Perform RNase digestion

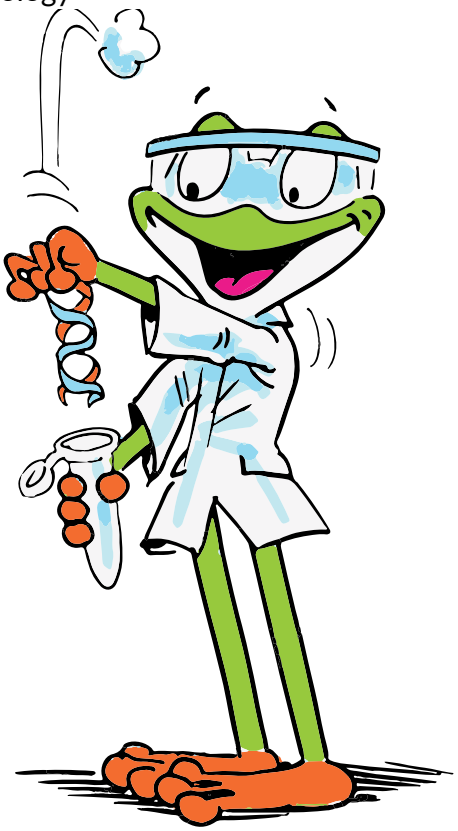
Related Products

Other XXprep Kits:

5482 XXprep Kit for DNA Fragments (from PCR reactions & Agarose gels)
4427 XXprep Kit for Plasmid DNA
6780 XXprep Kit for Total RNA (from Tissue, Cells, Bacteria)

Molecular biology chemicals and solutions:

1110 Agarose Basic for molecular biology
1057 Agarose low EEO for molecular biology
2276 Agarose low melt for molecular biology
1530 DNA Ladder 100 bp (lyophilized) for molecular biology
1531 DNA Ladder 1 kb (lyophilized) for molecular biology
1121 DNase I for molecular biology
2209 D-PBS (1X) w/o Ca and Mg (pH 7.4) for molecular biology
1340 EDTA for molecular biology
1108 EDTA disodium salt dihydrate for molecular biology
1353 EDTA solution pH 8.0 (0.5 M) for molecular biology
1131 Ethanol absolute for molecular biology
2324 Ethanol 80 % for molecular biology
2009 Ethanol 70 % for molecular biology
1254 Ethidium bromide solution 0.07 % dropping bottle for electrophoresis
1280 Glycerol anhydrous for molecular biology
1073 Glycerol 87 % for molecular biology
1317 LB Agar powder according to Lennox for molecular biology
1321 LB Agar powder according to Miller for molecular biology
1256 Lysozym for molecular biology
1496 2-Propanol for molecular biology
1263 RNase A (DNase-free) for molecular biology
1458 TE buffer (100X) pH 8.0 for electrophoresis
1115 Tris for molecular biology
1999 Tris Puffer pH 8.0 (1 M) for molecular biology
2001 Tris Puffer pH 7.5 (1 M) for molecular biology
1328 Tris hydrochloride for molecular biology
1058 Water for molecular biology



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neoFroxx beyond – we get everything you need

You still did not find the right product? Contact us. Our product specialists will be happy to assist!

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