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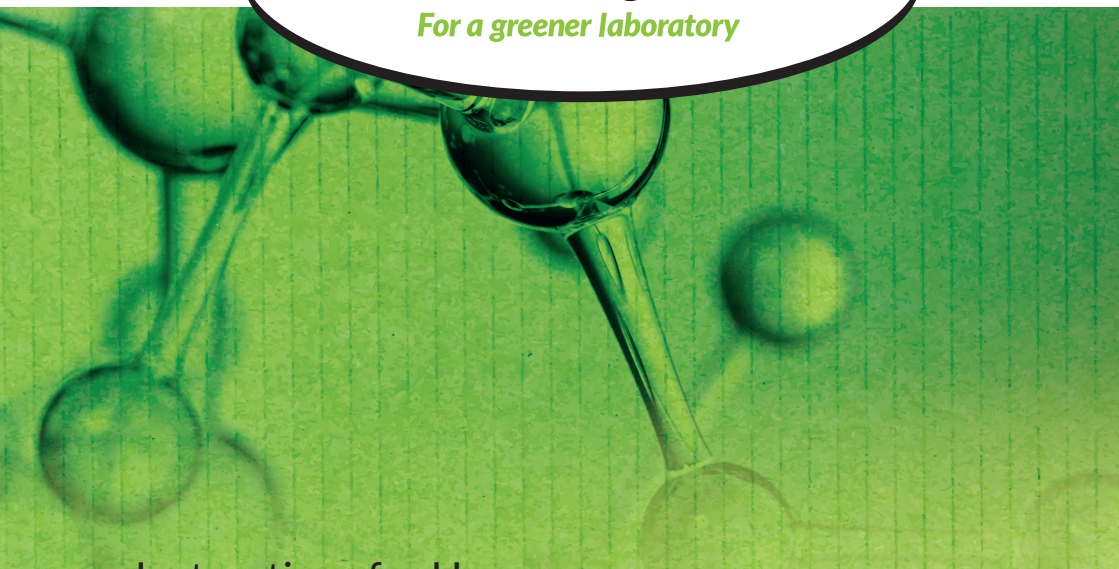

Instructions for Use

XXprep Kit for DNA Fragments (from PCR reactions & Agarose gels)

Published July 2021, TDS_EN_5482_XXprep-Kit-for-DNA-Fragments.
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.	
5482RC010	XXprep Kit for DNA Fragments (from PCR reactions & Agarose gels), 10 Reactions
5482RC050	XXprep Kit for DNA Fragments (from PCR reactions & Agarose gels), 50 Reactions
5482RC250	XXprep Kit for DNA Fragments (from PCR reactions & Agarose gels), 250 Reactions

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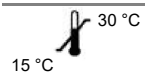
1 Introduction

1.1 Intended use

The XXprep Kit for DNA Fragments has been designed as a versatile kit for the extraction of DNA from agarose gels on the one hand and for the purification of PCR products from PCR reactions on the other hand. The kit uses a new patented chemistry for the purification of PCR products, which requires a minimum on washing steps resulting in a very fast and reliable purification of DNA fragments.

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> tests.
	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 device 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).
- Work 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 personnel in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order 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

The XXprep Kit for DNA Fragments 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. If there are any precipitates within the provided solutions solve these precipitates by careful warming. Or take care not to carry them over into the sample preparation procedure. Before every use make sure that all components have room temperature.

For further information see chapter “Kit components” (→ p. 7).

4 Functional testing and technical assistance

The neoFroxx GmbH guarantees the correct function of the kit for applications as described in the manual. This product has been 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 DNA Fragments or other neoFroxx 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.

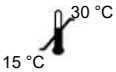
5 Product use and warranty

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 (→ “Intended use” p. 2) (→ “Product specifications” p. 9). Since the performance characteristics of our kits have not been validated for any specific application. neoFroxx 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 equivalents in other countries.

All products sold by the 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
For research use only!

6 Kit components



STORAGE CONDITIONS
All components are stored at room temperature.

	Σ 10	Σ 50	Σ 250
REF	5482RC010	5482RC050	5482RC250
Gel Solubilizer	8 ml	40 ml	180 ml
Binding Optimizer	1 ml	5 ml	15 ml
Binding Buffer	6 ml	30 ml	140 ml
Washing Solution LS (conc.)	3 ml	2 x 8 ml	2 x 40 ml
Elution Buffer	2 ml	2 x 2 ml	15 ml
Spin Filter	10	50	5 x 50
Receiver Tubes	10	50	5 x 50
Elution Tubes	10	50	5 x 50
Manual	1	1	1
Initial steps	Washing Solution LS Add 12 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!	Washing Solution LS Add 32 ml of 96-99.8 % ethanol to each bottle and mix thoroughly. Keep the bottle s always firmly closed!	Washing Solution LS Add 160 ml of 96-99.8 % ethanol to each bottle and mix thoroughly. Keep the bottles always firmly closed!

NOTE
Use only absolute/pure ethanol, NO methylated or denatured alcohol!

Components not included in the kit

- 1.5 ml or 2.0 ml reaction tubes
- 96–99.8 % ethanol

7 Product specifications

A Extraction of DNA from agarose gels

1. Starting material:
   - x TAE or TBE agarose gels (up to 300 mg)

2. Time for isolation:
   - x Approximately 20 minutes

3. Binding capacity and fragment length:
   - x Binding capacity: > 20 µg DNA
   - x Fragment length: 100 bp–30 kbp

4. Rate of recovery:
   - x 60–90 % (depending on the length of DNA amplicons)

B Purification of PCR products

1. Starting material:
   - x PCR reaction mixtures (up to 50 µl)

2. Time for extraction:
   - x 3 minutes (based on a new two-step procedure)

3. Binding capacity and fragment length:
   - x Binding capacity: > 20 µg DNA
   - x Fragment length: > 60 bp–30 kbp

4. Rate of recovery:
   - x 60–95 % (depending on the length of PCR fragments)

8 GHS classification

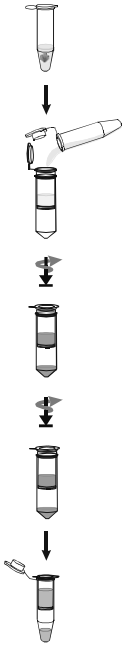
Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases	EUH
Gel Solubilizer	Guanidinium thiocyanate 50–100 % Acetic acid 1–<2.5 % Polyethylene glycol oc-tylphenol ether 0.3–<1.0 %	 Danger	302+312+ 332, 314, 412	101, 102, 103, 260,303+ 361+353, 305+351+ 338, 310, 405, 501	032
Binding Buffer	Propan-2-ol 50–100 % Diammonium hydro-gen 2-hydroxypro-pane-1,2,3-tri-carboxylate 0.1-<2.5 %	 Danger	225, 319, 336	101, 102, 103, 210, 261, 303+361+3 53, 305+351+3 38, 405, 501	
Binding Optimizer	Acetic acid 10–<25 %	 Warning	315, 319	101, 102, 103, 280,305+ 351+338, 362, 302+352, 403+233, 501	

CAUTION
DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

9 Recommended steps before starting

- Heat thermal mixer or water bath to 50 °C.
- Ensure that the Washing Solution LS has been prepared according to the instruction (→ "Kit components" p. 7).
- Centrifugation steps should be carried out at room temperature.
- Optionally, heat the needed amount of Elution Buffer to 50 °C. The final elution step with heated Elution Buffer will increase the DNA yield!

10 General procedure for DNA extraction from agarose gels



Gel solubilization

Binding of DNA onto Spin Filter

Washing of the bound DNA

Elution of DNA

11 Standard protocol: DNA extraction from agarose gel slices (TAE or TBE agarose gels)

NOTE
The standard protocol allows the elution of the bound DNA fragment with standard volumes of Elution Buffer (30–50 µl)!

IMPORTANT NOTE!
Optionally, heat the needed amount of Elution Buffer to 50 °C.
The final elution step with heated Elution Buffer will increase the DNA yield!

1. Excise the DNA fragment from the agarose gel with a sharp scalpel.

NOTE
Minimize the agarose gel slice. Check the weight. Do not use more than 300 mg gel slice for one Spin Filter!

2. Transfer the gel slice into a 1.5 ml or 2.0 ml reaction tube and add 650 µl Gel Solubilizer.
3. Incubate for 10 minutes at 50 °C until the agarose gel slice is completely dissolved.

NOTE
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 incubation.

4. Add 50 µl Binding Optimizer and mix the suspension by vortexing or pipetting sometimes up and down.

5. Apply the sample onto the Spin Filter located in a Receiver Tube. Close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute.

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

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

6. Open the Spin Filter and add 700 µl Washing Solution LS, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.
7. Repeat step 6 completely.
8. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
9. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add 30–50 µl Elution Buffer (optionally prewarmed to 50 °C). Incubate at room temperature for 1 minute. 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 Elution Buffer (depends on the expected yield of DNA). Elution with lower volumes of Elution Buffer increases the final concentration of DNA. Store the extracted DNA at 4–8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

12 "Mini Elute" protocol: DNA extraction from agarose gel slices (TAE or TBE agarose gels)

NOTE
The "Mini Elute" protocol allows the elution of the bound DNA fragment with low volumes of Elution Buffer (10–20 µl)!

IMPORTANT NOTE!
Optionally, heat the needed amount of Elution Buffer to 50 °C.
The final elution step with heated Elution Buffer will increase the DNA yield!

1. Excise the DNA fragment from the agarose gel with a sharp scalpel.

NOTE
Minimize the agarose gel slice. Check the weight. Do not use more than 300 mg gel slice for one Spin Filter!

2. Transfer the gel slice into a 1.5 ml or 2.0 ml reaction tube and add 650 µl Gel Solubilizer.
3. Incubate for 10 minutes at 50 °C until the agarose gel slice is completely dissolved.

NOTE
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 incubation.

4. Add 50 µl Binding Optimizer and mix the suspension by vortexing or pipetting sometimes up and down.

5. Apply the sample onto the Spin Filter located in a Receiver Tube. Close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute.

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

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

6. Open the Spin Filter and add 700 µl Washing Solution LS, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.
7. Repeat step 6 completely.
8. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
9. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add 10–20 µl Elution Buffer (optionally prewarmed to 50 °C). Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g (~11,000 rpm) for 1 minutes. A second elution step will increase the yield of extracted DNA.

   The recovery rate of the elution volume is approx. 9 µl in case of 10 µl of Elution Buffer.

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

13 General procedure for purification of PCR products

Addition of Binding Buffer to the PCR reaction mix

Binding of the PCR fragments onto Spin Filter

Elution of the PCR fragments

14 Standard protocol: Purification and concentration of PCR products from PCR reactions up to 50 µl

NOTE
The standard protocol allows the elution of the bound DNA fragment with standard volumes of Elution Buffer (20–50 µl)!

IMPORTANT NOTE!
Before starting with the purification procedure place a Spin Filter into a Receiver Tube.

Optionally, heat the needed amount of Elution Buffer to 50 °C.
The final elution step with heated Elution Buffer will increase the DNA yield!

A Binding of the PCR fragments

1. Add 500 µl Binding Buffer to the Spin Filter.
2. Add up to 50 µl of your PCR reaction mixture to the Spin Filter which is already pre-filled with the Binding Buffer.
3. Mix Binding Buffer and PCR reaction mixture by pipetting three times up and down. Don't destroy the filter membrane!

   Alternatively

   Mix 500 µl Binding Buffer with up to 50 µl of the PCR reaction mixture very well by pipetting or vortexing outside the Spin Filter in a separate reaction tube.

   After this transfer the mixed sample completely onto the Spin Filter.
4. Centrifuge for 3 minutes at 11,000 x g (~11,000 rpm). Discard the Receiver Tube.

NOTE
Avoid any contact of the Spin Filter with the flow through.

For maximum purity, discard the flow through after a first centrifugation step for 1 minute at 11,000 x g (~11,000 rpm) and place the Spin Filter back into the Receiver Tube and centrifuge for another 2 minutes at 11,000 x g (~11,000 rpm).

B Elution of the PCR fragments

1. Place the Spin Filter into an Elution Tube.
2. Pipette at least 20-50 µl Elution Buffer or RNase-free water directly onto the center of the Spin Filter.
3. Incubate for 1 minute at room temperature.
4. Centrifuge for 1 minute at 11,000 x g (~11,000 rpm). The Elution Tube now contains the purified PCR fragments.

NOTE
To increase the final DNA yield we recommend an extended incubation time with Elution Buffer (up to 5 minutes), which will lead to a slightly higher final yield.

For concentration of PCR fragments, it is possible to perform the elution with a lower volume of Elution Buffer than the volume of the starting PCR mixture. The minimum volume is 10 µl.

15 "Mini Elute" protocol: Purification and concentration of PCR products from PCR reactions up to 50 µl

NOTE
The “Mini Elute” protocol allows the elution of the bound DNA fragment with standard volumes of Elution Buffer (10–20 µl)!

IMPORTANT NOTE!
Before starting with the purification procedure place a Spin Filter into a Receiver Tube.

Optionally, heat the needed amount of Elution Buffer to 50 °C.
The final elution step with heated Elution Buffer will increase the DNA yield!

A Binding of the PCR fragments

1. Add 500 µl Binding Buffer to the Spin Filter.
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16 Troubleshooting

Problem / probable cause	Comments and suggestions
Low recovery / poor quality	
Incorrect Washing Solution LS or no ethanol added	Prepare the Washing Solution LS exactly as described in the manual. Store the Washing Solution with firmly fixed cap
Poor elution of DNA	Add the Elution Buffer directly onto the center of the Spin Filter (even if a small elution volume is used).
Ineffective solubilization of the agarose gel slice	The gel slice must be completely dissolved.
No Binding Optimizer added	Add the amount of Binding Optimizer needed to the solubilized suspension.
Poor quality of DNA	After binding of DNA an additional washing step with 80 % ethanol is recommended. Only use pure/non denatured ethanol. Add 600 µl of 80 % ethanol to the Spin Filter and centrifuge for 1 minute at 11,000 x g. Discard the flow through. For removing the ethanol centrifuge for 2 minutes at 11,000 x g. Continue with the elution step as described.
Problems with downstream application, e.g. ligation	
Contamination with salt components	Wash the Spin Filter as described in the manual.
Contamination with agarose traces	Wash the Spin Filter once with Gel Solubilizer (Gel extraction).
Contamination of the final DNA with ethanol	Keep the given centrifugation time, extend it if necessary (test the smell).
Problems with mineral oil	Take a higher volume of Binding Buffer.

Related Products

Other XXprep Kits:

2710 XXprep Kit for genomic DNA (from Blood, Tissue, Cells, Bacteria)
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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You still did not find the right product? Contact us. Our product specialists will be happy to assist!

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