

Life science unlimited

Manual



innuPREP PCRpure Kit

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This documentation describes the state at the time of publishing. It needs not necessarily agree with future versions. Subject to change!

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Contents

- 1 Safety precautions 3**
- 2 Storage conditions 3**
- 3 Function testing and technical assistance..... 3**
- 4 Product use and warranty 3**
- 5 Kit components 4**
- 6 General Scheme 4**
- 7 Product specifications..... 5**

- 8 Protocol: Purification and concentration of PCR products from
PCR reactions up to 50 µl 6**

- 9 Troubleshooting 8**

1 Safety precautions

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.

2 Storage conditions

The innuPREP PCRpure Kit should be stored dry, at room temperature (14–25 °C). It is stable for at least 12 months under these conditions. Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming.

3 Function testing and technical assistance

The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual. The components of each innuPREP PCRpure Kit were tested in purification of PCR fragments of different length.

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 innuPREP PCRpure Kit or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. For other countries please contact your local distributor.

4 Product use and warranty

The user is responsible to validate the performance of the Analytik Jena AG kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. Analytik Jena AG 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 Analytik Jena AG 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!

5 Kit components

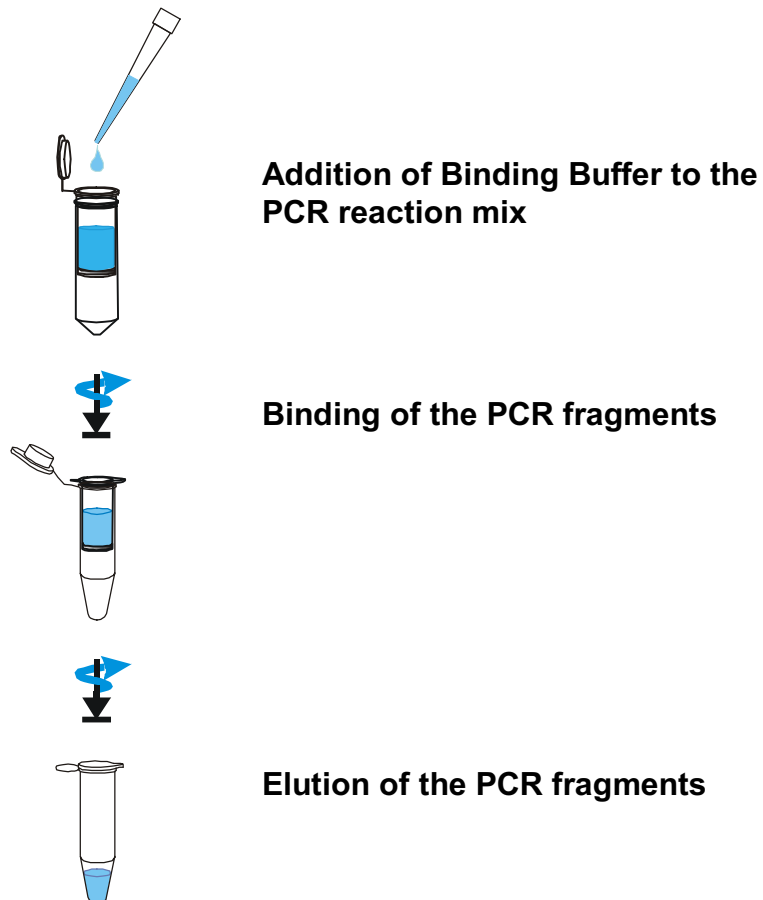


Important

Store all components at room temperature.


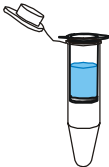
	10 purifications	50 purifications	250 purifications
Binding Buffer	10 ml	50 ml	250 ml
Elution Buffer	2 ml	5 ml	30 ml
Spin Filter	10	50	5 x 50
Receiver Tubes	10	50	5 x 50
Elution Tubes	10	50	5 x 50
Manual	1	1	1


6 General Scheme



innuPREP PCRpure Kit

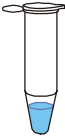
Protocol: Purification and concentration of PCR products


-
1. Starting material
- PCR reaction mix
 - Up to 50 μ l
-
2. Binding of PCR fragments
- 




- Add Spin Filter to Receiver Tube
 - Add 500 μ l Binding Buffer to Spin Filter
 - Add PCR reaction mix to Spin Filter
 - Mix: up and down pipetting

Alternative:

 - Add 500 μ l Binding Buffer to PCR reaction mix, Vortex
 - Add Spin Filter to Receiver Tube
 - Add mixed sample to Spin Filter
 - 10.000 x g (12.000 rpm): 2 min
-
3. Elution of PCR fragments
- 



- Add Spin Filter to an Elution Tube
 - Add minimum 10 μ l Elution Buffer (to center of the Spin Filter)
 - Incubation: 1 min @ RT
 - 6.000 x g (8.000 rpm): 1 min
-

Order No.:

845-KS-5010010	10 reactions
845-KS-5010050	50 reactions
845-KS-5010250	250 reactions

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7 Product specifications

1. Starting material:

PCR reaction mixtures (up to 50 µl)

2. Time for isolation:

- 3 minutes
- Based on a new two-step procedure

3. Typical yield:

60 bp – 30 kbp

4. Rate of recovery:

- 75 – 95 %
- Depending on the length of the PCR fragments

8 Protocol: Purification and concentration of PCR products from PCR reactions up to 50 µl



Important

Before starting with the purification procedure place a Spin Filter into a 2.0 ml Receiver Tube.

1. Binding of the PCR fragments

- Add **500 µl Binding Buffer** to the Spin Filter.
- Add up to **50 µl** of your **PCR reaction mixture** to the Spin Filter which is already pre-filled with the Binding Buffer.
- 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 sample** very well by pipetting or vortexing outside the Spin Filter in a separate reaction tube.
- After this transfer the mixed sample completely onto a Spin Filter.
- Centrifuge for 2 minutes at 10.000 x g (12.000 rpm). Discard the Receiver Tube.

Note: Avoid any contact of the Spin Filter with the flow through.

2. Elution of the PCR fragments

- Place the Spin Filter into a Elution Tube.
- Pipette **at least 10 µl Elution Buffer** (or ddH₂O) directly onto the center of the Spin Filter.
- Incubate for 1 minute at room temperature.
- Centrifuge for 1 minute at 6.000 x g (8.000 rpm). The Elution Tube now contains the purified PCR fragments.

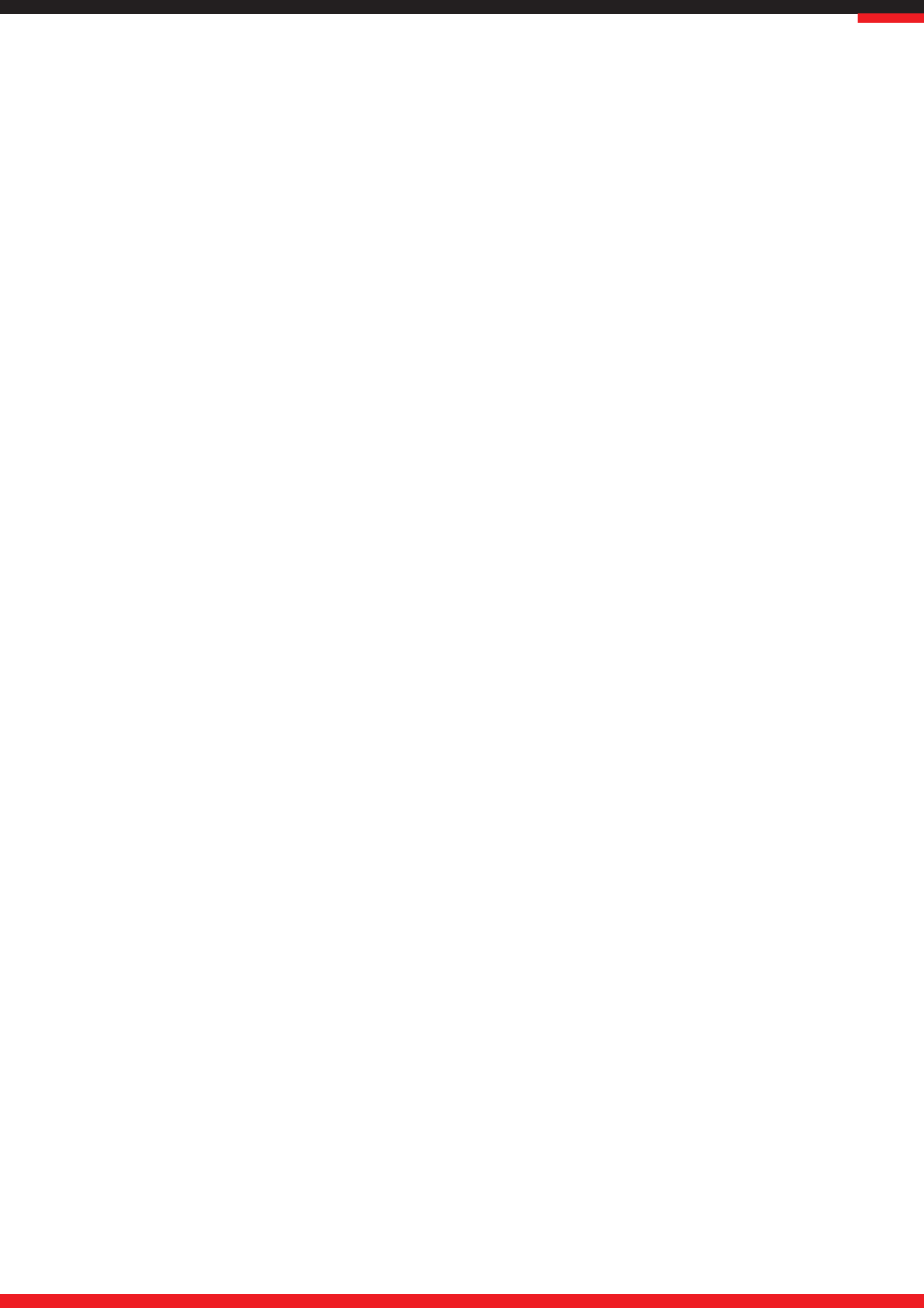


Important notes

1. 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.
 2. 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.
 3. If the volume of the PCR reaction mix is higher than 50 µl, split the PCR mix and add to each part 500 µl Binding Buffer. Load both mixes one after another (successively) on the Spin Filter. Centrifuge the first part for 1 minute and discard the filtrate. Centrifuge the second part of the mix for 2 minutes. Follow now the elution step as described above.
-

9 Troubleshooting

Problem / probable cause	Comments and suggestions
<p>Low recovery</p> <ul style="list-style-type: none"> • Poor elution of DNA • Problems with mineral oil 	<p>Add the Elution Buffer directly onto the center of the Spin Filter (even if a small elution volume is used).</p> <p>Apply the correct centrifugation steps.</p> <p>Take a higher volume of Binding Buffer.</p>



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