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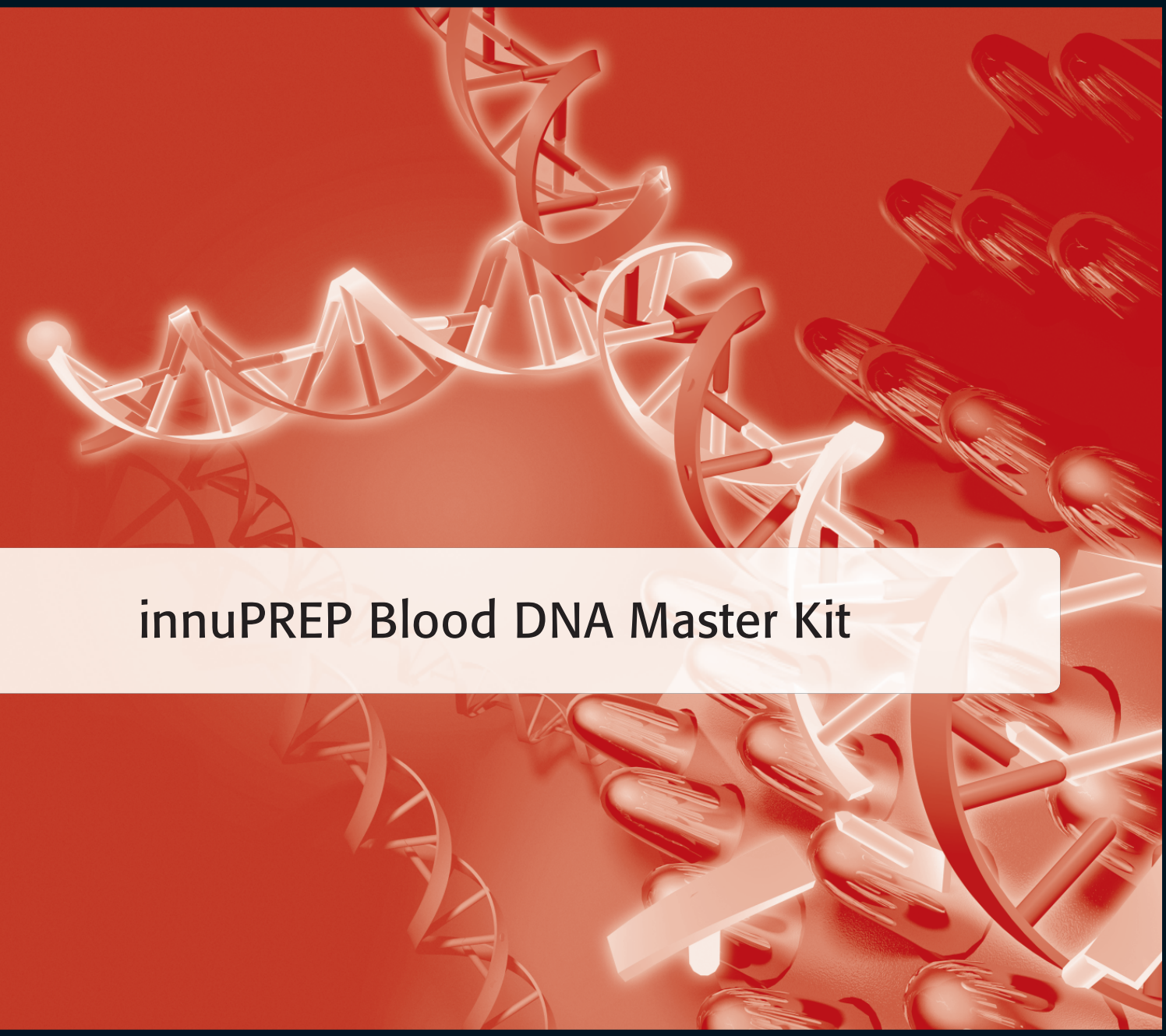
Manual



innuPREP Blood DNA Master Kit

Order No.:

845-KS-1070010	10 reactions
845-KS-1070050	50 reactions
845-KS-1070250	250 reactions

Publication No.: HB_KS-1070_e_120116

Manufacturer:
AJ Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin
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Contents

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 Blood DNA Master Kit should be stored dry, at room temperature (14-25 °C) and 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 Blood DNA Master Kit were tested by isolation of genomic DNA from whole blood samples and subsequent target amplification.

We reserve the right to change or modify our products to enhance there performance and design. If you have any questions or problems regarding any aspects of the innuPREP Blood DNA Master 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
All components are stored at room temperature.

	10 extractions	50 extractions	250 extractions
Ery Lysis Solution A	60 ml	2 x 130 ml	2 x 650 ml
Ery Lysis Solution B	60 ml	2 x 130 ml	2 x 650 ml
Lysis Tube PLP	10	50	5 x 50
Precipitation Buffer	1 ml	3 x 2 ml	25 ml
Washing Solution MS	3 ml (final volume 10 ml)	15 ml (final volume 50 ml)	60 ml (final volume 200 ml)
Elution Buffer	7 ml	30 ml	2 x 80 ml
Spin Filter (blue)	10	50	5 x 50
Receiver Tubes (2.0 ml)	30	3 x 50	15 x 50
Elution Tubes (1.5 ml)	10	50	5 x 50
Manual	1	1	1
Initial steps	• Add 7 ml of 96-99.8 % ethanol to the bottle Washing Solution MS, mix thoroughly and keep the bottle always firmly closed!	• Add 35 ml of 96-99.8 % ethanol to the bottle Washing Solution MS, mix thoroughly and keep the bottle always firmly closed!	• Add 140 ml of 96-99.8 % ethanol to the bottle Washing Solution MS, mix thoroughly and keep the bottle always firmly closed!

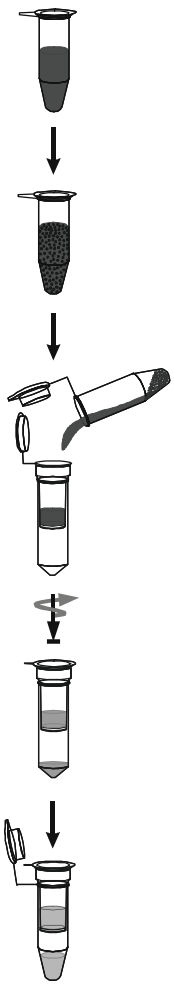
6 Recommended steps before starting

- Heat thermal mixer or water bath at 70 °C
- Ensure that the Washing Solution MS has been prepared according to the instruction (→ "Kit components" p. 4)
- Centrifugation steps should be carried out at room temperature
- Avoid freezing and thawing of starting material

7 Components not included in the kit

- 1.5 ml tubes
- 2.0 ml tubes or 15 ml (Falcon) tubes
- 96-99.8 % ethanol
- 70 % ethanol
- ddH_2O

8 General procedure for DNA extraction



Lysis of erythrocytes, following collecting of lymphocytes and lysis of lymphocytes

Removing of proteins

Binding of genomic DNA onto Spin Filter (blue)

Washing of the bound DNA

Elution of DNA

9 Product specifications

1. **Starting material:**
   - Fresh or frozen whole blood (0.5 – 5.0 ml)
   - Stabilized with EDTA or citrate

2. **Time for isolation:**
   Approximately 30 – 40 minutes

3. **Typical yield:**
   - 10 – 100 µg
   - Depending on initial volume of whole blood

4. **Binding capacity:**
   Approx.: 100 µg

5. **Purity (Ratio A_{260}:A_{280}):**
   1.7 – 2.0

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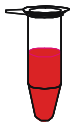
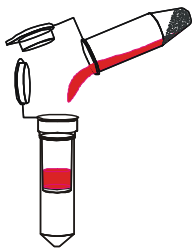
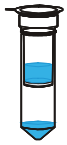
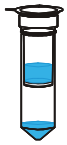
innuPREP Blood DNA Master Kit

Protocol 1: DNA isolation from whole blood sample

Recommended steps before starting
- Heat thermal mixer or water bath (70 °C)
- Prepare Washing Solution MS according to the instruction

1.	Starting material			▪ 0.5 – 1.0 ml
2.	Lysis of erythrocytes	▪ 2.0 ml tube		▪ Add 1 ml Ery Lysis Sol. A ▪ Vortex: short ▪ Incubation: 15 min @ RT ▪ 3.000 x g (~6.000 rpm): 3 min ▪ Discard supernatant ▪ Add 1 ml Ery Lysis Sol. B ▪ Vortex: 10 sec ▪ 2.000 x g (~5.000 rpm): 2 min
3.	Re-suspension of cell pellet			▪ Discard supernatant ▪ Add 200 µl ddH_2O ▪ Vortex
4.	Lysis of lymphocytes			▪ Add sample to Lysis Tube PLP ▪ Vortex: 5 sec ▪ Incubation: 70 °C, 20 min
5.	Removing of proteins			▪ Add 75 µl Precipitation Buffer ▪ Vortex: 10 sec ▪ Centrifuge: max speed, 3 min ▪ Add supernatant to a 1.5 ml tube
6.	Binding of DNA			▪ Add 500 µl 70 % ethanol ▪ Vortex ▪ Add Spin Filter to Receiver Tube ▪ Add sample to Spin Filter ▪ 10.000 x g (~12.000 rpm): 2 min

✂ Cut at the scattered line and laminate the card for a more convenient handling on the table top ✂

7.	Washing New Receiver Tube		▪ Add 750 µl MS ▪ 10.000 x g (~12.000 rpm): 1 min
8.	Remove Ethanol New Receiver Tube		▪ Discard filtrate ▪ Add Spin Filter to Receiver Tube ▪ Centrifuge: max speed, 2 min
9.	Elution (Repeat 2x)		▪ Add Spin Filter to an Elution Tube ▪ Add 200 µl Elution Buffer ▪ Incubation: 3 min @ RT ▪ 8.000 x g (~10.000 rpm): 1 min

Cut at the scattered line and laminate the card for a more convenient handling on the table top

Order No.:	845-KS-1070010	10 reactions
	845-KS-1070050	50 reactions
	845-KS-1070250	250 reactions

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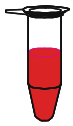
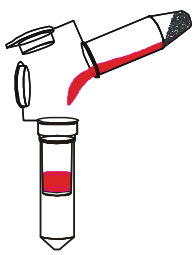
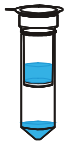
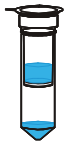
innuPREP Blood DNA Master Kit

Protocol 2: DNA isolation from whole blood sample

Recommended steps before starting
- Heat thermal mixer or water bath (70 °C)
- Prepare Washing Solution MS according to the instruction

1.	Starting material		▪ 2.0 – 5.0 ml
2.	Lysis of erythrocytes	▪ 2.0 ml tube	▪ Add 5 ml Ery Lysis Sol. A ▪ Vortex: short ▪ Incubation: 15 min @ RT ▪ 3.000 x g (~6.000 rpm): 3 min ▪ Discard supernatant ▪ Add 5 ml Ery Lysis Sol. B ▪ Vortex: 10 sec ▪ 2.000 x g (~5.000 rpm): 2 min
3.	Resuspension of cell pellet		▪ Discard supernatant ▪ Add 200 µl ddH_2O ▪ Vortex
4.	Lysis of lymphocytes		▪ Add sample to Lysis Tube PLP ▪ Vortex: 5 sec ▪ Incubation: 70 °C, 30 min
5.	Removing of proteins		▪ Add 75 µl Precipitation Buffer ▪ Vortex: 10 sec ▪ Centrifuge: max speed, 3 min ▪ Add supernatant to a 1.5 ml tube
6.	Binding of DNA		▪ Add 500 µl 70 % ethanol ▪ Vortex ▪ Add Spin Filter to Receiver Tube ▪ Add sample to Spin Filter ▪ 10.000 x g (~12.000 rpm): 2 min

✂ Cut at the scattered line and laminate the card for a more convenient handling on the table top ✂

7.	Washing New Receiver Tube		▪ Add 750 µl MS ▪ 10.000 x g (~12.000 rpm): 1 min
8.	Remove Ethanol New Receiver Tube		▪ Discard filtrate ▪ Add Spin Filter to Receiver Tube ▪ Centrifuge: max speed, 2 min
9.	Elution (Repeat 2x)		▪ Add Spin Filter to an Elution Tube ▪ Add 300 µl Elution Buffer ▪ Incubation: 3 min @ RT ▪ 8.000 x g (~10.000 rpm): 1 min

Cut at the scattered line and laminate the card for a more convenient handling on the table top

Order No.:

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10 Protocol 1: DNA isolation from whole blood sample (0.5 - 1.0 ml)

1. Transfer **0.5-1.0 ml** of **whole blood** into a 2.0 ml reaction tube. Add **1 ml** of **Ery Lysis Solution A**, vortex shortly and incubate at room temperature for 15 minutes. Centrifuge at 3.000 x g (~6.000 rpm) for 3 minutes. Remove and discard the supernatant very carefully.

   Don't discard the pellet!

2. Add **1 ml** of **Ery Lysis Solution B**. Vortex the sample for 10 seconds. Centrifuge at 2.000 x g (~5.000 rpm) for 2 minutes. Remove the supernatant very carefully.

   Don't discard the pellet!

3. Add **200 µl ddH_2O** to the cell pellet and re-suspend the cell pellet completely by pipetting up and down or by vigorous vortexing.

   Important Note: The cell pellet must be completely re-suspended.

4. Transfer the re-suspended sample into a Lysis Tube PLP. Vortex for 5 seconds and incubate the Lysis Tube PLP at 70 °C for 20 minutes.

   Note: We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3-4 times during lysis time. No shaking will reduce the lysis efficiency. Incomplete lysis of the pellet will reduce the final yield!

5. After lysis add **75 µl** of **Precipitation Buffer**, mix vigorously by pulsed vortexing for 10 seconds.

   Note: The solution has to become milky! If not, vortex longer! Optionally, incubation of the tube on ice for 1 minute following the vortexing step might be useful.

   Centrifuge the 2.0 ml tube at max. speed for 3 minutes. Transfer the clear supernatant into a new 1.5 ml tube. Avoid contamination with precipitates.

6. Add **500 µl** of **70 % ethanol** to the sample, mix by vortexing or by pipetting up and down several times.

   Note: It is important that the sample and the ethanol are mixed vigorously to get a homogeneous solution.

7. Apply the sample to the Spin Filter (blue) 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.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

8. Open the Spin Filter and add **750 µl Washing Solution MS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

9. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

10. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **200 µl Elution Buffer**. Incubate at room temperature for 3 minutes. Centrifuge at 8.000 x g (~10.000 rpm) for 1 minute. Repeat the elution step **once again** with **200 µl Elution Buffer**.

    Note: If the Elution Buffer has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Note

The DNA can be eluted with a lower or a higher volume of Elution Buffer (depends on the expected yield of genomic DNA). Elution with lower volumes of Elution Buffer increases the final concentration of DNA. Store the extracted DNA at +4 °C. For long time storage placing at –20 °C is recommended.

11 Protocol 2: DNA isolation from whole blood sample (2.0 - 5.0 ml)

1. Transfer **2.0-5.0 ml** of **whole blood** into a 15 ml reaction tube (Falcon tube). Add **5 ml** of **Ery Lysis Solution A**, vortex shortly and incubate at room temperature for 15 minutes. Centrifuge at 3.000 x g (~6.000 rpm) for 3 minutes. Remove and discard the supernatant very carefully.

   Don't discard the pellet!

2. Add **5 ml** of **Ery Lysis Solution B**. Vortex the sample for 10 seconds. Centrifuge at 2.000 x g (~5.000 rpm) for 2 minutes. Remove the supernatant very carefully.

   Don't discard the pellet!

3. Add **200 µl ddH_2O** to the cell pellet and re-suspend the cell pellet completely by pipetting up and down or by vigorous vortexing.

   Important Note: The cell pellet must be completely re-supended.

4. Transfer the re-suspended sample into a Lysis Tube PLP. Vortex for 5 seconds and incubate the Lysis Tube PLP at 70 °C for 30 minutes.

   Note: We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3-4 times during lysis time. No shaking will reduce the lysis efficiency. Incomplete lysis of the pellet will reduce the final yield!

5. After lysis add **75 µl** of **Precipitation Buffer**, mix vigorously by pulsed vortexing for 10 seconds.

   Note: The solution has to become milky! If not, vortex longer! Optionally, incubation of the tube on ice for 1 minute following the vortexing step might be useful.

   Centrifuge the 2.0 ml tube at max. speed for 3 minutes. Transfer the clear supernatant into a new 1.5 ml tube. Avoid contamination with precipitates.

6. Add **500 µl** of **70 % ethanol** to the sample, mix by vortexing or by pipetting up and down several times.

   Note: It is important that the sample and the ethanol are mixed vigorously to get a homogeneous solution.

7. Apply the sample to the Spin Filter (blue) 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.

Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

8. Open the Spin Filter and add **750 µl Washing Solution MS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
9. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
10. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **300 µl Elution Buffer**. Incubate at room temperature for 3 minutes. Centrifuge at 8.000 x g (~10.000 rpm) for 1 minute. Repeat the elution step **once again** with **300 µl Elution Buffer**.

    Note: If the Elution Buffer has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Note

The DNA can be eluted with a lower or a higher volume of Elution Buffer (depends on the expected yield of genomic DNA). Elution with lower volumes of Elution Buffer increases the final concentration of DNA. Store the extracted DNA at +4 °C. For long time storage placing at –20 °C is recommended.

12 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
• Insufficient lysis and/or too much starting material or incomplete resuspension of cell pellet	Increase lysis time. Increase centrifugation speed. Reduce amount of starting material. Resuspend the cell pellet completely.
Low amount of extracted DNA	
• 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 70 % ethanol	Mix sample with 70 % ethanol by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted DNA	
• Too much Elution Buffer	Elute the DNA with lower volume of Elution Buffer
Degraded or sheared DNA	
• Incorrect storage of starting material	Avoid thawing of the material.
• Old material	Old material often contains degraded DNA.

engl. 01/12 – Analytik Jena AG, Jena

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