Instructions for Use
Life Science Kits & Assays

innuPREP DNA Micro Kit

analytikjena
An Endress+Hauser Company

Order No.:
845-KS-1011010 10 reactions
845-KS-1011050 50 reactions

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Contents

1 Introduction

1.1 Intended use

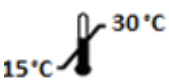
The kit has been designed as a tool for very fast and efficient isolation of genomic DNA from small amounts of different types of starting materials like micro biopsies, whole blood up to 50 µl, blood sticks and from limited amounts of cells. The extraction procedure is based on a new kind of chemistry, which combines an extremely fast lysis step with a subsequent efficient binding of genomic DNA on a Spin Filter surface following washing of the bound DNA and finally eluting of the DNA. The recovery of DNA and the quality are excellent.

Extracted DNA is available approx. 8 minutes after lysis of starting material. The isolated DNA is suitable for all downstream applications commonly used.

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.

Symbol	Information
	**Expiry date**
	**Lot number** The number of the kit charge.
	**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. 2).
- 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 immediately with a large amount of water.

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 in order to avoid any injuries. Analytik Jena AG has not tested the liquid waste generated during using the kit for potential residual infectious components. This case is highly unlikely, but cannot be excluded completely. 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 on GHS classification please download the Safety Data Sheet (SDS) from our website (www.analytik-jena.com). Find the SDS of each product and its components in the "downloads"-section.

3 Storage conditions

The Kit is shipped at ambient temperature.

Upon arrival, store lyophilized **Proteinase K** at 4 °C to 8 °C. Aliquot dissolved **Proteinase K** and store at -22 °C to -18 °C. Repeated freezing and thawing will reduced activity dramatically!

All other components of the innuPREP DNA Micro Kit 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 Function testing and technical assistance

The Analytik Jena AG 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 there performance and design. If you have any questions or problems regarding any aspects of the innuPREP DNA Micro 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.

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 intended use (→ "Product specifications" p. 7).
All plastic components and the chemistry are disposable products. When changing the starting material or the flow trace, no guarantee of the operability is issued. Since the performance characteristics of Analytik Jena AG kits have just been validated for the application described above, the user is responsible for the validation of the performance of Analytik Jena AG kits using other protocols then those described below. 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 equivalent regulations required 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
The kit is for reseach use only!

6 Kit components

6.1 Included kit components

	Σ 10	Σ 50
REF	845-KS-1011010	845-KS-1011050
Lysis Solution TLS	2 x 2 ml	15 ml
Binding Solution TBS	2 x 2 ml	15 ml
Proteinase K	for 1 x 0.3 ml working solution	for 1 x 1.5 ml working solution
Washing Solution HS (conc.)	3 ml	15 ml
Washing Solution MS (conc.)	3 ml	15 ml
Elution Buffer	2 ml	10 ml
Spin Filter	10	50
Receiver Tubes	30	3 x 50
Elution Tubes	10	50
Manual	1	1

6.2 Components not incuded in the kit

- 1.5 ml tubes
- 96–99.8 % ethanol (molecular biology grade, undenaturated)
- ddH2O for dissolving Proteinase K
- 2.0 ml tubes; optional
- RNase A (100 mg/ml); optional
- Xylene or Octan; optional

7 Initial steps before starting

- Heat thermal mixer or water bath at 50 °C or 70 °C (for blood samples), following 90 °C (for paraffin samples)

- Add to Proteinase K the indicated amount of ddH_2O to each vial, mix thoroughly and store as described above.

845-KS-1011010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-1011050	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add to **Washing Solution HS (conc.)** the indicated amount of absolute ethanol. Mix thoroughly and store as described above.

845-KS-1011010	Add 3 ml ethanol to 3 ml Washing Solution HS (conc.).
845-KS-1011050	Add 15 ml ethanol to 15 ml Washing Solution HS (conc.).

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

845-KS-1011010	Add 7 ml ethanol to 3 ml Washing Solution MS (conc.).
845-KS-1011050	Add 35 ml ethanol to 15 ml Washing Solution MS (conc.)

- Centrifugation steps should be carried out at room temperature.
- Avoid freezing and thawing of starting material.

8 Product specifications

1. Starting material:

- Small pieces of tissue samples or biopsies (up to 5 mg)
- Paraffin embedded tissue samples
- Eucaryotic cells (up to 1x106 cells)
- Whole blood samples (up to 50 µl) and blood sticks

2. Time for isolation:

- Approximately 8 minutes after lysis step

3. Typical yield:

- Depends on type and amount of starting material
- Binding capacity of the spin column is > 100 µg gDNA

9 Protocols for isolation of genomic DNA

9.1 Protocol 1: Isolation from tissue samples or biopsies

1. Cut **max. 5 mg of tissue** sample or biopsies into small pieces and place the tissue in a common reaction tube (1.5 ml or 2.0 ml). Add **200 µl Lysis Solution TLS and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 sec. Incubate at 50 °C for 30 minutes (or until the sample is completely lysed).

NOTE
We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively vortex the sample during incubation some times. No shaking will reduce the lysis efficiency!

2. Centrifuge the tube at 11,000 x g (~11,000 rpm) for 1 minute to spin down unlysed material. Transfer the supernatant into a new 1.5 ml tube.

NOTE
To remove RNA from the sample (if necessary) add 4 µl of RNase A solution (100 mg/ ml), vortex shortly and incubate 5 min at room temperature.

3. Add **200 µl Binding Solution TBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

IMPORTANT
Please be careful! Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. But it is important that the sample and the Binding Solution TBS are mixed completely 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 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.

5. Open the Spin Filter and add **400 µl Washing Solution HS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 30 sec. Discard the 2.0 ml Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
6. Open the Spin Filter and add **750 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 30 sec. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
7. Centrifuge at maximum speed for 2 min to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
8. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **50-100 µl Elution Buffer**. 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 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.

9.2 Protocol 2: Isolation from paraffin embedded tissue sample

1. Place a piece of starting material into a 2.0 ml reaction tube, add **1 ml Octane or Xylene** and vortex carefully to dissolve the paraffin. Follow the dissolution until the tissue sample looks transparent (while paraffin remains white).
2. Centrifuge at max. speed for 5 min at room temperature. Discard the supernatant very carefully using a pipette.

IMPORTANT
Do not remove the pellet!

NOTE
This step should be repeated if any paraffin is still in the sample.

3. Add **1 ml ethanol** (96−99.8 %) to the pellet and vortex vigorously.

4. Centrifuge at max. speed at room temperature for 3 minutes and remove the ethanol by pipetting.

IMPORTANT
Do not remove the pellet!

5. Repeat the washing step with ethanol once again.
6. Incubate the open tube at 37 °C for 10–15 minutes to evaporate the residual ethanol completely.
7. Add **200 µl Lysis Solution TLS and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 sec. Incubate at 50 °C until the sample is completely lysed.
8. Pre-heat the thermal mixer without the sample to 90 °C, afterwards incubate the lysed sample for 60 minutes at 90 °C
9. Add **200 µl Binding Solution TBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

IMPORTANT
Please be careful! Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. But it is important that the sample and the Binding Solution TBS are mixed completely to get a homogeneous solution.

10. Apply the sample to the Spin Filter located in a 2.0 ml 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.

11. Open the Spin Filter and add 400 µl Washing Solution HS, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 30 sec. Discard the 2.0 ml Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
12. Open the Spin Filter and add 750 µl Washing Solution MS, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 30 sec. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

13. Centrifuge at maximum speed for 2 min to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

14. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add 50-100 µl Elution Buffer. 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
We recommend for the elution step the use of 50−100 µl of Elution Buffer because of the expected lower yield of DNA. Store the extracted DNA at 4 °C. For long time storage placing at −20 °C is recommended.

9.3 Protocol 3: Isolation from cell cultures

1. Pellet cells by centrifugation for 10 min at 5.000 x g (7.500 rpm). Discard supernatant. Add **200 µl Lysis Solution TLS and 20 µl Proteinase K** to the pellet, mix vigorously by pulsed vortexing for 5 sec. Incubate at 50 °C until the sample is completely lysed.

NOTE
We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively vortex the sample during incubation some times. No shaking will reduce the lysis efficiency!

NOTE
To remove RNA from the sample (if necessary) add 4 µl of RNase A solution (100 mg/ ml), vortex shortly and incubate 5 min at room temperature.

2. Add **200 µl Binding Solution TBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

IMPORTANT
Please be careful! Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. But it is important that the sample and the Binding Solution TBS are mixed completely to get a homogeneous solution.

3. Apply the sample to the Spin Filter located in a 2.0 ml 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.

4. Open the Spin Filter and add **400 µl Washing Solution HS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 30 sec. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
5. Open the Spin Filter and add **750 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 30 sec. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
6. Centrifuge at maximum speed for 2 min to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
7. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **50-100 µl Elution Buffer**. 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 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.

9.4 Protocol 4: Isolation from whole blood samples

1. Pipette **50 µl of blood** sample (or less, at least 1 µl) into a 1.5 ml reaction tube.
2. Add **250 µl Lysis Solution TLS and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 sec. Incubate at 70 °C for 10 minutes.

NOTE

We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively vortex the sample during incubation some times. No shaking will reduce the lysis efficiency!

NOTE

To remove RNA from the sample (if necessary) add 4 µl of RNase A solution (100 mg/ ml), vortex shortly and incubate 5 min at room temperature.

3. Add **200 µl Binding Solution TBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

IMPORTANT

Please be careful! Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. But it is important that the sample and the Binding Solution TBS are mixed completely 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 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.

5. Open the Spin Filter and add **400 µl Washing Solution HS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 30 sec. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
6. Open the Spin Filter and add **750 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 30 sec. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
7. Centrifuge at maximum speed for 2 min to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

8. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **50-100 µl Elution Buffer**. 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 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.

9.5 Protocol 5: Isolation from blood sticks

1. Place the blood stick into a 1.5 ml reaction tube. Add **200 µl Lysis Solution TLS and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 sec. Incubate at 50 °C for 30 min.

NOTE
We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively vortex the sample during incubation some times. No shaking will reduce the lysis efficiency!

2. Add **200 µl Binding Solution TBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

IMPORTANT
Please be careful! Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. But it is important that the sample and the Binding Solution TBS are mixed completely to get a homogeneous solution.

3. Apply the sample to the Spin Filter located in a 2.0 ml 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.

4. Open the Spin Filter and add **400 µl Washing Solution HS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 30 sec. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
5. Open the Spin Filter and add **750 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 30 sec. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
6. Centrifuge at maximum speed for 2 min to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
7. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **50-100 µl Elution Buffer**. 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 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.

10 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient lysis and/or too much starting material	Increase lysis time. Increase centrifugation speed. After lysis centrifuge the lysate to pellet un-Lysed material. Reduce amount of starting material.
Low amount of extracted DNA	
Insufficient lysis	Increase lysis time! Reduce amount of starting material. Over loading 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 TBS	Mix sample with Binding Solution TBS by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted DNA	
Too much Elution Buffer was used in the elution step	Elute the DNA with lower volume of Elution Buffer
Degraded or sheared DNA	
Incorrect storage of starting mate-rial	Ensure that the starting material is frozen immediately after taking in liquid nitrogen or at -18 ℃ to -22° C! For long time storage continuously store at -78 ℃ to -82° C! Avoid thawing of the material.
Old starting material	Old material often contains degraded DNA. Repeat with fresh material.
RNA contamination	
Extracted DNA is contami-nated with RNA	Perform an RNase A digestion.

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