Instructions for Use
Life Science Kits & Assays

innuPREP DNA Mini Kit

analytikjena
An Endress+Hauser Company

Order No.:
845-KS-1041010 10 reactions
845-KS-1041050 50 reactions
845-KS-1041250 250 reactions

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Manufacturer:
AJ Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin · Germany
Made in Germany!

Distribution/Publisher:
Analytik Jena AG
Konrad-Zuse-Straße 1
07745 Jena · Germany

Phone +49 3641 77 9400
Fax +49 3641 77 767776
www.analytik-jena.com
info@analytik-jena.com

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1 Introduction

1.1 Intended use

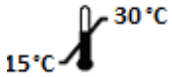
The innuPREP DNA Mini Kit has been designed as a very efficient tool for fast isolation of genomic DNA from different amounts and different types of starting materials like tissue samples up to 50 mg, paraffin-embedded tissue material, buccal swabs, mouse or rodent tail, eukaryotic cell pellets. The kit is intended for use by professional users. 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. 15 minutes after lysis of starting material. The isolated DNA is suitable for a wide range of different downstream applications like amplification reactions and further analytical procedures. Diagnostic results generated using the extraction procedure in conjunction with diagnostic tests should be interpreted with regard to other clinical or laboratory results. To reduce irregularities in diagnostic results, internal controls for downstream applications should be 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	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> reactions.

Symbol	Information
	**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 charge.
	**Manufactured by** Contact information of manufacturer.
	**For single use only** Do not use components for a second time.

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 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 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

Store lyophilized **Proteinase K** at 4 °C to 8 °C! Divide dissolved **Proteinase K** into aliquots and store at -22 °C to -18 °C. Repeated freezing and thawing will reduce the activity dramatically!

All other components of the innuPREP DNA Mini 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 Functional testing and technical assistance

The Analytik Jena AG 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 innuPREP DNA Mini 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 manual (→ “Product specifications“ p. 7). 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 than 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 equivalents in other countries.

All products sold by 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 research use only!

6 Kit components

6.1 Included kit components

	Σ 10	Σ 50	Σ 250
REF	845-KS-1041010	845-KS-1041050	845-KS-1041250
Lysis Solution TLS	5 ml	25 ml	120 ml
Binding Solution TBS	5 ml	25 ml	120 ml
Proteinase K	for 1 x 0.3 ml working solution	for 1 x 1.5 ml working solution	for 5 x 1.5 ml working solution
Washing Solution HS (conc.)	3 ml	15 ml	70 ml
Washing Solution MS (conc.)	3 ml	15 ml	60 ml
Elution Buffer	2 x 2 ml	15 ml	60 ml
Spin Filter	10	50	5 x 50
Receiver Tubes	40	4 x 50	20 x 50
Elution Tubes	10	50	5 x 50
Manual	1	1	1

6.2 Components not included in the kit

- Xylene or Octan (for paraffin embedded tissue samples)
- 1.5 ml and 2.0 ml tubes
- ddH_2O for dissolving **Proteinase K**
- 96–99.8 % ethanol (molecular biology grade, undenaturated)
- RNase A (10 mg/ml); optional

7 Product specifications

1. **Starting material:**

- Tissue samples (up to 50 mg)
- Rodent tail (up to 1 cm)
- Paraffin embedded tissue samples
- Buccal swabs
- Eukaryotic cells (up to 5 x 10^6 cells)

2. **Time for isolation:**

- Approximately 8 minutes after lysis step

3. **Typical yield:**

- Depends on type and amount of starting material
- The extracted genomic DNA (gDNA) can be used for a wide range of different molecular biology applications.
- Binding capacity of the spin column is > 100 µg gDNA

8 Initial steps before starting

- Heat thermal mixer or water bath (paraffin embedded samples: 37 °C, followed by 50 °C and 90 °C; buccal swab/ cell cultures: 50 °C).
- Add to Proteinase K the indicated amount of ddH_2O to each vial, mix thoroughly and store as described above.

845-KS-1041010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-1041050	Add 1.5 ml ddH_2O to lyophilized Proteinase K.
845-KS-1041250	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-1041010	Add 3 ml ethanol to 3 ml Washing Solution HS (conc.).
845-KS-1041050	Add 15 ml ethanol to 15 ml Washing Solution HS (conc.).
845-KS-1041250	Add 70 ml ethanol to 70 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-1041010	Add 7 ml ethanol to 3 ml Washing Solution MS (conc.).
845-KS-1041050	Add 35 ml ethanol to 15 ml Washing Solution MS (conc.).
845-KS-1041250	Add 140 ml ethanol to 60 ml Washing Solution HS (conc.).

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

9 Protocols for Isolation of genomic DNA

9.1 Protocol 1: Isolation from tissue samples or rodent tails

1. Cut max. 50 mg of tissue sample or up to 1 cm of rodent tail into small pieces and place the tissue in a 1.5 ml or 2.0 ml reaction tube. Add **400 µl Lysis Solution TLS and 25 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds Incubate at 50 °C until the sample is completely lysed (approx. 1–2 hours; especially for rodent tails use not more than 2 hours for lysis).

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 every 10 minutes during the incubation. No shaking will reduce the lysis efficiency.

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

NOTE
To remove RNA from the sample (if necessary) add 2 µl of RNase A solution (10 mg/ml), vortex shortly and incubate for 5 minute at RT.

3. Add **400 µl Binding Solution TBS** to the lysed sample, mix by brief vortexing for 15 seconds.

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 Receiver Tube. Close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 2 minutes. 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.

5. Open the Spin Filter and add **500 µl Washing Solution HS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 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 1 minute. Dis-

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

7. Centrifuge at 11,000 x g (~11,000 rpm) for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.
8. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **200 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 to 8 °C. For long time storage placing at −18 °C to -22 °C is recommended.

9.2 Protocol 2: Isolation from paraffin embedded tissue samples

1. Place a piece of starting material into a 2.0 ml 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 maximum speed for 5 minutes at room temperature. Discard the supernatant very carefully by aspirating with 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 maximum 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 **400 µl Lysis Solution TLS** and **25 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds 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 **400 µl Binding Solution TBS** to the lysed sample, mix by brief vortexing for 15 seconds.

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 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.

11. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
12. Open the Spin Filter and add **500 µl Washing Solution HS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
13. 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 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

14. Centrifuge at 11,000 x g (~11,000 rpm) for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.
15. Place the Spin Filter into an 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
Store the extracted DNA at 4 °C to 8 °C, for long time storage at -18 °C to -22 °C.

9.3 Protocol 3: Isolation from buccal swab

IMPORTANT
To get maximum yield of DNA it is essential to leave the swab during the complete lysis time in the 1.5 ml tube. It is possible to cut the shaft of the swab, so that you can close the cap of the tube. The removal of the swab from the tube ahead of time will lead to a dramatically reduced final yield!

1. Place the swab into a 1.5 ml reaction tube. Add **400 µl Lysis Solution TLS and 25 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds. Incubate at 50 °C for 10–15 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 3-4 times during the incubation. No shaking will reduce the lysis efficiency.

2. After lysis time remove the swab from the tube and squeeze the swab on the wall of the tube to remove all **Lysis Solution TLS** from the swab.
3. Add **400 µl Binding Solution TBS** to the lysed sample, mix by brief vortexing for 15 seconds.

IMPORTANT
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 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.

5. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
6. Open the Spin Filter and add **500 µl Washing Solution HS**, close the cap and centrifuge at 11,000 x g (~11,000 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 **750 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
8. Centrifuge at 11,000 x g (~11,000 rpm) for 3 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 **200 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 to 8 °C. For long time storage placing at -18 °C to -22 °C is recommended.

9.4 Protocol 4: Isolation from cell cultures

1. Pellet up to 5 x 10^6 cells by centrifugation for 10 minutes at 5,000 x g (~5,000 rpm). Discard supernatant. Add **400 µl Lysis Solution TLS** and **25 µl Proteinase K** to the pellet, mix vigorously by pulsed vortexing for 5 seconds. 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 3-4 times during the incubation. No shaking will reduce the lysis efficiency.

2. Add **400 µl Binding Solution TBS** to the lysed sample, mix by brief vortexing for 15 seconds.

IMPORTANT
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 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.

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

5. Open the Spin Filter and add **500 µl Washing Solution HS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 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 1 minute. Dis-

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

7. Centrifuge at 11,000 x g (~11,000 rpm) for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.
8. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **200 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 to 8 °C. For long time storage placing at -18 °C to -22 °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 unlysed material. Reduce amount of starting material.
Low amount of extracted DNA	
Insufficient lysis	Increase lysis time! Reduce amount of starting material. Overloading 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 material	Ensure that the starting material is frozen immediately after taking in liquid nitrogen or at -18 °C to -22° C! For long time storage continuously store at -78 °C 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 contaminated with RNA	Perform an RNase A digestion.

engl. 08 / 19 – Analytik Jena AG, Jena

Headquarters

Analytik Jena AG
Konrad-Zuse-Str. 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279
info@analytik-jena.com
www.analytik-jena.com

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