Instructions for Use
Life Science Kits & Assays

innuPREP Forensic Kit

analytikjena
An Endress+Hauser Company

Order No.:
845-KS-1051010 10 reactions
845-KS-1051050 50 reactions
845-KS-1051250 250 reactions

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CONTENTS

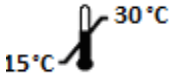
1 Introduction

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 forensic samples like, hairs or hair roots; stains of blood, saliva or sperm; finger nails; cigarette butts; bubble gum; buccal swabs; stamps and envelopes as well as fingerprints on different surfaces. The kit is intended for use by professional users. The kit has been designed to be used for a wide range of different downstream application 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.

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.
	**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. 3).
- 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, please ask for the Safety Data Sheet (SDS).

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 Forensic 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.

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

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 Forensic 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

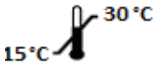
The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Intended use“ p. 2), (→ “Product specifications“ p. 9). Since the performance characteristics of Analytik Jena AG kits have just been validated for the application 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

IMPORTANT
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 reduce the activity dramatically!



STORAGE CONDITIONS
All other components are stored at room temperature.

	Σ 10	Σ 50	Σ 250
REF	845-KS-1051010	845-KS-1051050	845-KS-1051250
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 ml	10 ml	30 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

	Σ 10	Σ 50	Σ 250
Initial steps	**Proteinase K** Dissolve Proteinase K by addition of 0.3 ml ddH_2O, mix thoroughly and store as described above! **Washing Solution HS** Add 3 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed! **Washing Solution MS** Add 7 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!	**Proteinase K** Dissolve Proteinase K by addition of 1.5 ml ddH_2O, mix thoroughly and store as described above! **Washing Solution HS** Add 15 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed! **Washing Solution MS** Add 35 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!	**Proteinase K** Dissolve Proteinase K by addition of 1.5 ml ddH_2O, mix thoroughly and store as described above! **Washing Solution HS** Add 70 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed! **Washing Solution MS** Add 140 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!

COMPONENTS NOT INCLUDED IN THE KIT

- RNase A (10 mg/ml); optional
- 1 M DTT solution
- 1.5 ml reation tubes
- ddH_2O for dissolving **Proteinase K**
- 96–99.8 % ethanol, non-denatured or methylated

7 Product specifications

1. Starting material:
   - Swabs from different surfaces (e.g. cups, bottles, fingerprints)
   - Blood samples
   - Sperm samples
   - Hair, hair roots or barb hairs
   - Envelopes
   - Finger nails
   - Cigarette butts or paper
   - Chewing gum

2. **Time for isolation:**
   - Approximately 15 minutes after lysis step

NOTE

Using the kit for other kinds of forensic sample which are not described in the protocols, the selection of one of the described protocols is recommended.

NOTE

Optionally, for the isolation of DNA from forensic samples containing extremely low amounts of DNA it could be helpful to add **Carrier RNA** after lysis to the Binding Step.

We recommend to use Carrier RNA (e.g. Poly(A) RNA; Roche Diagnostics; No.108626). Dissolve the RNA in RNase-free water to obtain a solution of 1 µg/µl. Divide into aliquots and store at -22 °C to -18 °C.

Do not freeze and thaw the aliquots more than 3 times.

We recommend the addition of **1 µl Carrier RNA** per sample.

8 GHS classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases	EUH
Binding Solution TBS	Propan-2-ol 25-50 % Polyethylene glycol octylphenol ether 25-50 %	 Danger	225, 315, 318, 336, 411	101, 102, 103, 210, 303+361+353, 305+351+338, 310, 405, 501	
Washing Solution HS (conc.)	Guanidinium thiocyanate 50-100 %	 Danger	302, 314, 412	101, 102, 103, 260, 303+361+353, 305+351+338, 310, 405, 501	032
Proteinase K	Proteinase, engyodontium album 50-100 %	 Danger	315, 317, 319, 334, 335	101, 102, 103, 261, 280, 305+351+338, 342+311, 405, 501	

8.1 Hazard phrases

225	Highly flammable liquid and vapour.
302	Harmful if swallowed.
314	Causes severe skin burns and eye damage.
315	Causes skin irritation.
317	May cause an allergic skin reaction.
318	Causes serious eye damage.
319	Causes serious eye irritation.
334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
335	May cause respiratory irritation.
336	May cause drowsiness or dizziness.
411	Toxic to aquatic life with long-lasting effects.

412	Harmful to aquatic life with long lasting effects.

8.2 Precaution phrases

101	If medical advice is needed, have product container or label at hand.
102	Keep out of reach of children.
103	Read label before use.
210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
260	Do not breathe dust/fume/gas/mist/vapors/spray.
261	Avoid breathing dust/fume/gas/mist/vapors/spray.
280	Wear protective gloves/protective clothing/ eye protection/face protection.
310	Immediately call a POISON CENTER/doctor.
405	Store locked up.
501	Dispose of contents/container in accordance with local/regional/national/international regulations.
342+311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
303+361+353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
305+351+338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

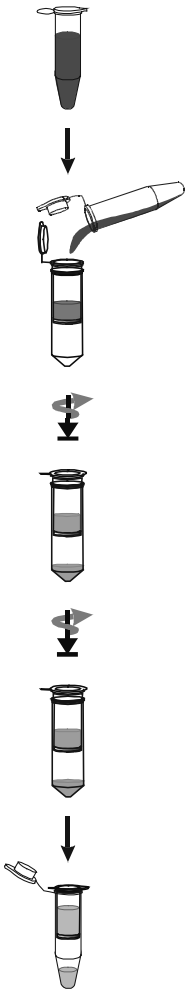
8.3 EU hazard statements

032	Contact with acids liberates very toxic gas.

9 Recommended steps before starting

- Ensure that the **Proteinase K / Washing Solution HS / Washing Solution MS** have been prepared according to the instruction (→ "Kit components" p. 7).
- Heat thermal mixer or water bath at 50 °C (optional 42 °C).
- Centrifugation steps should be carried out at room temperature.
- Avoid freezing and thawing of starting material.

10 General procedure for DNA extraction



Lysis of starting material

Binding of DNA on Spin Filter

Washing of bound DNA

Elution of DNA

11 Protocols

11.1 Protocol 1: DNA isolation from buccal swab samples

NOTE

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 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.

   After lysis 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.

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.

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.

2. Add **400 µl Binding Solution TBS** to the lysed sample, mix by brief vortexing or by pipetting up and down several times.

NOTE
It is important that the sample and the Binding Solution TBS are mixed vigorously 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.

   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.

4. 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.

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 1 minute.

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

6. Centrifuge at 11,000 x g (~11,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.

7. 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

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.

11.2 Protocol 2: DNA isolation from buccal swab samples from different surfaces (cups, bottles, fingerprints etc.)

NOTE

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 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.

   After lysis 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.

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.

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.

2. Add **400 µl Binding Solution TBS** to the lysed sample, mix by brief vortexing or by pipetting up and down several times.

NOTE
It is important that the sample and the Binding Solution TBS are mixed vigorously 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.

   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.

4. 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.

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 1 minute.

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

6. Centrifuge at 11,000 x g (~11,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.

7. 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

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.

11.3 Protocol 3: DNA isolation from blood stains, saliva stains, stamps and envelopes, etc.

1. Cut the material containing the stains into small pieces and transfer it into a 1.5 ml reaction tube.
   Add **400 µl Lysis Solution TLS** and **25 µl Proteinase K**.

   For semen stains add **30 µl 1 M DTT** (not provided) to the Lysis Solution/ Proteinase K mix.

   Mix vigorously by pulsed vortexing for 5 seconds. Incubate at 50 °C for at least 2 hours.

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.

3. Add **400 µl Binding Solution TBS** to the lysed sample, mix by brief vortexing or by pipetting up and down several times.

NOTE

It is important that the sample and the Binding Solution TBS are mixed vigorously 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.

   Discard 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 2 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 **30 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.

11.4 Protocol 4: DNA extraction from hair roots, barb hairs, finger nails, etc.

1. Cut the material into small pieces and transfer it into a 1.5 ml reaction tube.
   Add **400 µl Lysis Solution TLS** and **25 µl Proteinase K**.
2. Add **30 µl 1 M DTT** (not provided) to the Lysis Solution / Proteinase K mix. Mix vigorously by pulsed vortexing for 5 seconds.
   Incubate at 50 °C for at least 2 hours (incubation overnight at 42 °C is also possible).

   Assure that the hair roots are in the Lysis Solution during the lysis time!

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.

3. 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.
4. Add **400 µl Binding Solution TBS** to the lysed sample, mix by brief vortexing or by pipetting up and down several times.

NOTE

It is important that the sample and the Binding Solution TBS are mixed vigorously to get a homogeneous solution.

5. 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.

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 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 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.

11.5 Protocol 5: DNA extraction from cigarette butts

1. Remove of a small piece (3–5 mm) of the brown filter paper or of a part of the filter and place the material in a 1.5 ml reaction tube. Add **400 µl of Lysis Solution TLS** and **25 µl of Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds.

   Incubate at 50 °C for at least 2 hours (incubation overnight at 42 °C is also possible).

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.

3. Add **400 µl Binding Solution TBS** to the lysed sample, mix by brief vortexing or by pipetting up and down several times.

NOTE

It is important that the sample and the Binding Solution TBS are mixed vigorously 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. Discard 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 2 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 **30 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 (with other 30 µl Elution Buffer) 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.

11.6 Protocol 6: DNA extraction from chewing gum

1. Cut a part of the chewing gum into small pieces and place the material 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 at least 2 hours (incubation overnight at 42 °C is also possible).

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.

3. Add **400 µl Binding Solution TBS** to the lysed sample, mix by brief vortexing or by pipetting up and down several times.

NOTE

It is important that the sample and the Binding Solution TBS are mixed vigorously 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.

   Discard 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 2 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 **30 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 (with other 30 µl Elution Buffer) could 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.

11.7 Protocol 7: DNA extraction from tissue samples

1. Cut the material (fresh or frozen) into small pieces and transfer it 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 at least 1 hour (incubation overnight at 42 °C is also possible).

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 or by pipetting up and down several times.

NOTE

It is important that the sample and the Binding Solution TBS are mixed vigorously 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.

   Discard 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 2 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 **30 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 (with other 30 µl Elution Buffer) could 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.

12 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.

13 Related products

Name	Amount	Order No.
Products for PCR & Gel Electrophoresis		
innuPREP DOUBLEpure Kit	10 rxn	845-KS-5050010
	50 rxn	845-KS-5050050
	250 rxn	845-KS-5050250
innuPREP Gel Extraction Kit	10 rxn	845-KS-5030010
	50 rxn	845-KS-5030050
	250 rxn	845-KS-5030250
innuPREP PCRpure Kit	10 rxn	845-KS-5010010
	50 rxn	845-KS-5010050
	250 rxn	845-KS-5010250
innuTaq DNA Polymerase (5 U/µl)	500 U	845-EZ-1000500
50x inNucleotide Mix (1.5 mM)	2x 0.5 ml	845-AS-9000100
inNucleotide Set (100 mM)	4x 0.25 ml	845-AS-1100250
innuMIX rapidPCR MasterMix	100 rxn	845-AS-1600100
	200 rxn	845-AS-1600200
innuMIX Standard PCR MasterMix	100 rxn	845-AS-1700100
	200 rxn	845-AS-1700200
innuMIX Green PCR MasterMix	100 rxn	845-AS-1400100
	200 rxn	845-AS-1400200
innuSTAR 100 bp DNA Ladder Express	500 µl	845-ST-1010100
	5x 500 µl	845-ST-1010500
innuSTAR 1 kb DNA Ladder Express	500 µl	845-ST-1020100
	5x 500 µl	845-ST-1020500
6x Loading Dye Bromophenol Blue	3x 1.0 ml	845-ST-3010003
	6x 1.0 ml	845-ST-3010006

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