Instructions for Use
Life Science Kits & Assays

innuPREP Forensic Kit

Innuscreen GmbH

Order No.:

845-KS-1051010 10 reactions
845-KS-1051050 50 reactions
845-KS-1051250 250 reactions

Publication No.: HB_KS-1051_e_211026

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1 Introduction

1.1 Intended use

The **innuPREP Forensic 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; fingernails; 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 regarding 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 before 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 and the kit

For easy reference and orientation, the manual and labels use 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.
15 °C 30 °C	**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	**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. 3).
- Working steps are numbered.

2 Safety precautions

NOTE

Read through this chapter carefully before 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. IST Innuscreen GmbH 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 and the Safety Data Sheet (SDS) please contact sds.info@ist-ag.com.

3 Storage conditions

The kit is shipped at ambient temperature.

Upon arrival, store lyophilized and dissolved **Proteinase K** at 4 °C to 8 °C.

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.

4 Functional testing and technical assistance

The IST Innuscreen GmbH 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 IST Innuscreen GmbH products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 30 9489 3380. 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. 8). Since the performance characteristics of IST Innuscreen GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen GmbH 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 IST Innuscreen GmbH 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-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

6.2 Components not included in the kit

- 1 M DTT solution (for Protocols 3 and 4)
- 1.5 ml reaction tubes
- ddH_2O for dissolving **Proteinase K**
- 96–99.8 % ethanol (molecular biology grade, undenatured)
- RNase A (10 mg/ml); optional
- Carrier RNA (1 vial sufficient for 1 ml solution, 31-00278)

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 and before the Binding step. We recommend the addition of **1 µl Carrier RNA** per sample.

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
- Fingernails
- 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 samples containing extremely low amounts of DNA we recommend using our **Carrier RNA (**→ "Kit components", p.7

8 Initial steps before starting

- Heat thermal mixer or water bath to 50 °C (optional 42 °C).
- Add the indicated amount of ddH_2O to each vial **Proteinase K**, mix thoroughly and store as described above.

845-KS-1051010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-1051050	Add 1.5 ml ddH_2O to lyophilized Proteinase K.
845-KS-1051250	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

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

845-KS-1051010	Add 3 ml ethanol to 3 ml Washing Solution HS (conc.).
845-KS-1051050	Add 15 ml ethanol to 15 ml Washing Solution HS (conc.).
845-KS-1051250	Add 70 ml ethanol to 70 ml Washing Solution HS (conc.).

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

845-KS-1051010	Add 7 ml ethanol to 3 ml Washing Solution MS (conc.).
845-KS-1051050	Add 35 ml ethanol to 15 ml Washing Solution MS (conc.).
845-KS-1051250	Add 140 ml ethanol to 60 ml Washing Solution MS (conc.).

- Centrifugation steps should be carried out at room temperature.

9 Protocols for isolation of DNA from forensic samples

9.1 Protocol 1: Isolation from buccal swab samples

NOTE
To achieve maximum yield of DNA it is essential to leave the swab in the 1.5 ml tube for the entire lysis time. It is possible to cut off the shaft of the swab so that the cap of the tube can be closed. 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 using 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 minutes 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.

9.2 Protocol 2: Isolation from buccal swab samples from different surfaces (cups, bottles, fingerprints etc.)

NOTE
To achieve maximum yield of DNA it is essential to leave the swab in the 1.5 ml tube for the entire lysis time. It is possible to cut off the shaft of the swab so that the cap of the tube can be closed. 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 using 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 minutes 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.

9.3 Protocol 3: 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** (→ Components not included in the kit, p. 7) 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 using 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.

9.4 Protocol 4: Isolation from hair roots, barb hairs, fingernails, 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** (→ Components not included in the kit, p. 7) 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).

IMPORTANT NOTE
Assure that hair roots are covered by Lysis Solution during lysis.

NOTE
We recommend using 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.

9.5 Protocol 5: Isolation 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 using 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.

9.6 Protocol 6: Isolation 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 using 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.

9.7 Protocol 7: Isolation 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 using 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 minutes 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.

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.

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