Instructions for Use
Life Science Kits & Assays

innuPREP Plant DNA Kit

Innuscreen GmbH

Order No.:
845-KS-1060010 10 reactions
845-KS-1060050 50 reactions
845-KS-1060250 250 reactions

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This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

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Contents

1. Introduction

1.1 Intended use

The innuPREP Plant DNA Kit has been designed for simple, reliable and fast isolation of genomic DNA (gDNA) from different kinds of plant material. The extraction procedure is based on a new kind of patented technology (called DC chemistry). The innuPREP Plant DNA Kit is optimized for the rapid preparation of highly pure gDNA from plant material.

The protocol is based on using mortar and pestle and liquid nitrogen for homogenization. The homogenization is followed by a lysis step. For lysis of plant material, the kit contains three different Lysis Solutions. These are Lysis Solution SLS, Lysis Solution OPT and Lysis Solution CBV. All three Lysis Solutions contain a mixture of chaotropic and anti-chaotropic salts (DC-Technology), detergents and other additives. Following the homogenization, the lysates will be cleared by a centrifugation based precipitation and/or filtration using a Prefilter to remove polysaccharides, contaminations and residual cellular debris. The clear flow-through is mixed with Binding Solution SBS to create conditions for optimal binding of gDNA to the silica membrane of the Spin Filter. After washing steps, the gDNA can be eluted from the membrane by using Elution Buffer (provided) or RNase-free water (not provided) and is ready-to-use for subsequent downstream applications.

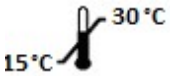
The innuPREP Plant DNA Kit allows processing of up to 50–100 mg (dry weight) or 120–180 mg (wet weight) starting material.

The kit has been tested for isolation of gDNA from leafs, fruits, woods, needles as well as seeds. The starting material can be fresh or frozen. Depending on the individual sample typical yields are in the range from 3-25 µg DNA (more yield is also possible).

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.
	**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. 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 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. 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 Plant DNA 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 kit was 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 Plant DNA 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 our kits have not 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 the 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-1060010	845-KS-1060050	845-KS-1060250
Lysis Solution SLS	5 ml	25 ml	120 ml
Lysis Solution OPT	5 ml	25 ml	120 ml
Lysis Solution CBV	5 ml	25 ml	120 ml
Precipitation Buffer P	2 ml	4 x 2 ml	30 ml
Binding Solution SBS	5 ml	15 ml	60 ml
Proteinase K	for 1 x 0.3 ml working solution	for 1 x 1.5 ml working solution	for 4 x 1.5 ml working solution
Washing Solution MS (conc.)	6 ml	24 ml	2 x 60 ml
Elution Buffer	2 x 2 ml	15 ml	60 ml
Prefilter	10	50	5 x 50
Spin Filter	10	50	5 x 50
Receiver Tubes	50	5 x 50	25 x 50
Elution Tubes	10	50	5 x 50
Manual	1	1	1

6.2 Components not included in the kit

- ddH_2O for dissolving Proteinase K
- 96–99.8 % ethanol (molecular biology grade, undenaturated)
- 1.5 ml tubes
- 2.0 ml tubes; optional
- RNase A (100 mg/ml); optional

7. Product specifications

1. Starting material:

- Up to 100 mg fresh, frozen or dried plant material
- 120 - 180 mg wet weight (if material is very wet/ contains more water)

NOTE
Avoid freezing and thawing of starting material. If plant tissue will not be used immediately after harvesting, it can be stored in liquid nitrogen, lyophilized/dried or frozen. Fresh material can be kept at 4 °C to 8 °C for 24 hours but should be frozen at -22 °C to -18 °C or for longer storage at -80 °C for later processing. Ground tissue powder can also be stored at -80 °C. Alternatively, tissue can be dried or lyophilized after harvesting to allow storage at room temperature (15 °C to 30 °C). To ensure DNA quality, samples should be completely dried within 24 hours of collection.

2. Time for isolation:

- Approximately 30–40 minutes

3. Binding capacity:

- 50 µg DNA

4. Typical yield:

- 3–25 µg (Depending on the kind and initial amount of the starting material)

8. Initial steps before starting

- Heat thermal mixer or water bath at 65 °C.
- Add the indicated amount of ddH_2O to **Proteinase K**, mix thoroughly and store as described above.

845-KS-1060010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-1060050	Add 1.5 ml ddH_2O to lyophilized Proteinase K.
845-KS-1060250	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated amount of absolute ethanol to each bottle of **Washing Solution MS (conc.)**, mix thoroughly. Store as described above.

845-KS-1060010	Add 14 ml ethanol to 6 ml Washing Solution MS (conc.).
845-KS-1060050	Add 56 ml ethanol to 24 ml Washing Solution MS (conc.).
845-KS-1060250	Add 140 ml ethanol to 60 ml Washing Solution MS (conc.).

- Centrifugation steps should be carried out at room temperature.

9. Homogenization and lysis of plant samples

It is recommended to collect young materials (e.g., leaves, needles) since they contain more cells per weight and therefore result in higher yields. In addition, young leaves and needles contain smaller amounts of polysaccharides and polyphenolics and are therefore easier to handle.

Complete and quick disruption of starting material is essential to ensure high DNA yields and to avoid DNA degradation. The lysis procedure is most effective with well-homogenized, powdered samples. Suitable methods include any type of commercial homogenizers (rotor-stator homogenizer) or bead mills (e.g. SpeedMill PLUS, Analytik Jena GmbH) using ceramic beads. However, we recommend grinding with a mortar and pestle in the presence of liquid nitrogen to obtain optimal yields. When using tissues other than leaves, the disruption method may require optimization to ensure maximum DNA yield and quality. After homogenization and treatment of the sample with lysis solution, the crude lysate can be cleared easily either with Prefilters or by centrifugation.

9.1 Disruption of plant material

9.1.1 Disruption by using a mortar and pestle

Use mortar and pestle to grind the plant material in the presence of liquid nitrogen. Freeze plant material in liquid nitrogen and be careful during homogenization, because do not let the sample thaw at any time. We recommend precooling the used laboratory equipment. Grind frozen plant sample to a fine powder and refill mortar with liquid nitrogen to keep the

sample frozen, if necessary. Use precooled tubes for sample storage until lysis step, but make sure no liquid nitrogen is transferred or all nitrogen has evaporated before closing the tube.

9.1.2 Disruption by using bead mill homogenizers

Use Lysis Tube P for plant material and leaves or Lysis Tube J for seeds, rice and needles. Pipette 50 µl ddH_2O to the plant material and vortex for about 30 seconds (e.g. SpeedMill PLUS, Analytik Jena GmbH). Repeat the chilling and vortexing procedure until the entire plant material is ground to a fine solution.

It is also possible to chill the tube in liquid nitrogen. After the homogenization, describe above, chill the tube once more and remove the beads by rolling them out gently or using a magnet. Keep the material frozen throughout the whole homogenization procedure. Do not add nitrogen to the tube since this leads to sticking and loss of plant material attached to the beads.

9.1.3 Disruption by using rotor-stator homogenizers

Rotor-stator homogenizers are only useful to disrupt soft plants in the presence of lysis solution. Keep homogenizer submerged at all times to reduce foaming.

9.2 Lysis of plant samples

Increasing the amount of starting material

The standard protocols of innuPREP Plant DNA Kit allow processing of 50–100 mg (dry weight) or 120–180 mg (wet weight) of plant material. This usually yields 1-25 µg of high quality DNA. However, the amount of DNA that can be expected per mg of sample depends on the size and ploidy of the genome.

To obtain sufficient DNA yield, it might be advantageous to process a higher than the recommended sample mass. However, to ensure a complete lysis, all lysis solution volumes of protocol step 2 have to be increased proportionally and require multiple loading steps.

9.3 Selecting the optimal lysis solution system

Plants are very heterogeneous and contain varying amounts of polyphenols, acidic components, or polysaccharides which can lead to suboptimal DNA extraction or performance in downstream applications. Therefore, three different lysis solutions are provided for optimal processing, purification performance, high yields and an excellent DNA quality for the most common plant species.

The standard protocol uses **Lysis Solution SLS**, containing CTAB as detergent component. Additionally, the SDS based **Lysis Solution OPT** is provided which requires subsequent precipitation step to remove all impurities by **Precipitation Buffer P**. For some plant species **Lysis Solutions SLS** and **OPT** can be used with similar results. In these cases, please make a choice for the easiest protocol. Further the **Lysis Solution CBV** has been optimized for isolation of gDNA from seeds but can also be used for other plant materials.

In order to find optimal lysis conditions when using a certain plant sample for the first time, it is recommended to do side-by-side preparations of one batch of homogeneously ground material with the three different lysis solutions and make a decision for the best one (regarding yield, quality or other relevant parameters). The following example of applica-

tion illustrates the effects of different lysis solutions on yield and quality of the extracted gDNA.

Application Example

Isolation of gDNA from rice (*Oryza sativa*) using the three different Lysis solutions SLS, OPT and CBV. The spectrophotometric measurement shows different results depending on the lysis solution used. It compares the Ct-values of the detection of a plant specific genome by Real-Time PCR.

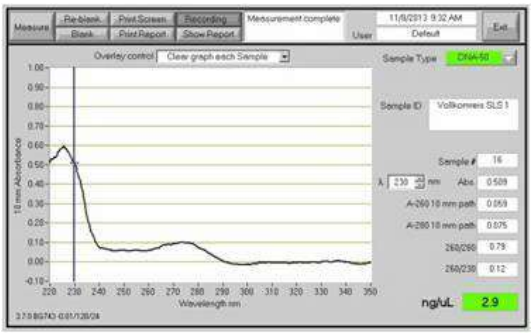


Fig. 1: Concentration of DNA with **Lysis Solution SLS**: 2.9 ng/µl and purity (A_{260}/A_{230}): 0.79.

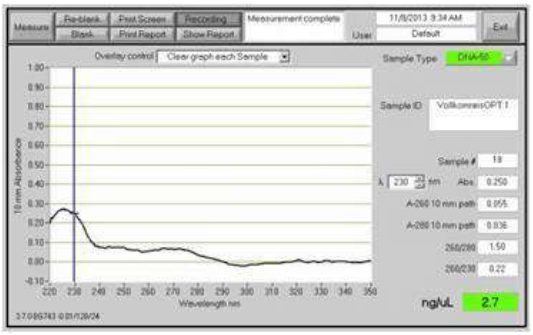


Fig. 2: Concentration of DNA with **Lysis Solution OPT**: 2.7 ng/µl and purity (A_{260}/A_{230}): 1.50.

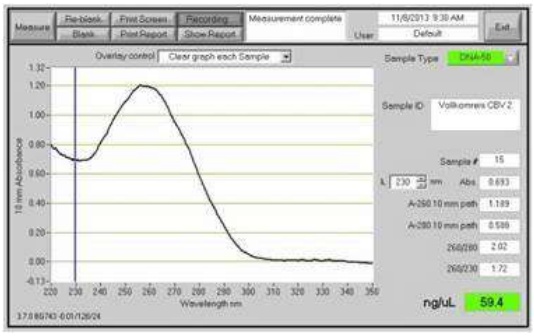


Fig. 3: Concentration of DNA with **Lysis Solution CBV**: 59.4 ng/µl and purity (A_{260}/A_{230}): 2.02.

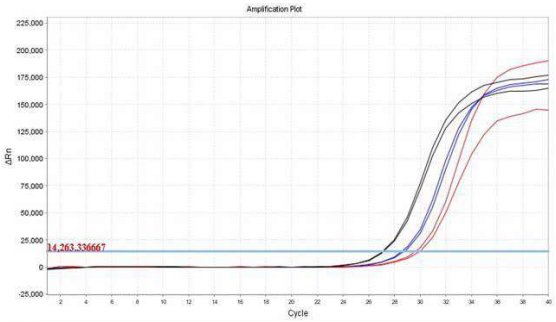


Fig. 4: Ct-values of Real-Time PCR with **Lysis Solution SLS** (red): 29.8, OPT (blue): 28.8 and CBV (black): 27.0.

IMPORTANT NOTE

For a large variety of plant species, either lysis solution generates good results.

10. Protocols for DNA isolation from plant material

IMPORTANT NOTE

The innuPREP Plant DNA Kit include three different lysis solutions for optimal results with most common plant species. Please refer to section "Selecting the optimal lysis solution system" (p.11) for choosing the optimal lysis solution system for your individual plant sample.

In order to prevent confusions when you perform several preparations in parallel, label the tubes and the filters!

1. Homogenization of about **50–100 mg** of **starting material** by a pestle under liquid N_2.

   Commercially available equipment for homogenization (e.g. SpeedMill PLUS) can also be used. Alternatively grinding of plant material with sand is also possible (→ "Homogenization and lysis of plant samples", p. 9).

NOTE

Use 120–180 mg of starting material if extraction from material which is very wet or contains more water.

2. Proceed with cell lysis using **Lysis Solution SLS** (→ Protocol 1, p. 13), **Lysis Solution OPT** (→ Protocol 2, p. 16) or **Lysis Solution CBV** (→ Protocol 3, p. 18).

10.1 Protocol 1: Extraction using Lysis Solution SLS

1. Transfer the plant powder or other homogenized starting material in a 1.5 ml or 2.0 ml reaction tube. Add **400 µl Lysis Solution SLS and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds.

   Incubate at 65 °C for approx. 30 minutes (longer incubation is also possible, up to 60 minutes).

NOTE

We recommend to use a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex

the sample optionally 3–4 times during lysis step. No shaking will reduce the lysis efficiency.

2. Transfer the sample onto a Prefilter located in a Receiver Tube and centrifuge the tube at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Prefilter.

   Don't discard the Receiver Tube with the filtrate!

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

3. Add **200 µl Binding Solution SBS** to the lysed sample (filtrate from step 3), mix by pipetting up and down several times.

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

4. Apply the sample to a Spin Filter located in a new 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. For higher sample volumes repeat the loading step.

5. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
6. Open the Spin Filter and add **650 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. Open the Spin Filter and add **650 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.

8. Centrifuge at max. speed 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 **100–200 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 (e.g. 100 µl + 100 µl) 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 -22 °C to -18 °C is recommended.

10.2 Protocol 2: Extraction using Lysis Solution OPT

1. Transfer the plant powder or other homogenized starting material in a 1.5 ml or 2.0 ml reaction tube. Add **400 µl Lysis Solution OPT**, mix vigorously by pulsed vortexing for 5 seconds.

   Incubate at 65 °C for approx.. 30 minutes (longer incubation is also possible, up to 60 minutes).

NOTE
We recommend to use a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during lysis step. No shaking will reduce the lysis efficiency.

2. Add **100 µl Precipitation Buffer P** and vortex the sample for 5 seconds. Incubate at room temperature for 5 minutes and centrifuge at maximum speed for 5 minutes.
3. Transfer the clear supernatant onto a Prefilter located in a Receiver Tube and centrifuge the tube at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Prefilter.

   Don't discard the Receiver Tube with the filtrate!

NOTE
If there is a pellet after centrifugation, don't discard the pellet. Transfer the supernatant carefully into a new reaction tube.

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

4. Add **200 µl Binding Solution SBS** to the lysed sample (filtrate from step 3), mix by pipetting up and down several times.

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

5. Apply the sample to a Spin Filter located in a new 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. For higher sample volumes repeat the loading step.

6. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
7. Open the Spin Filter and add **650 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. Open the Spin Filter and add **650 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.
9. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
10. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **100–200 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 (e.g. 100 µl + 100 µl) 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 -22 °C to -18 °C is recommended.

10.3 Protocol 3: Extraction using Lysis Solution CBV

1. Transfer the plant powder or other homogenized starting material in a 1.5 ml or 2.0 ml reaction tube. Add **400 µl Lysis Solution CBV and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds.

   Incubate at 65 °C for approx.. 30 minutes (longer incubation is also possible, up to 60 minutes).

NOTE
We recommend to use a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during lysis step. No shaking will reduce the lysis efficiency.

2. Add **100 µl Precipitation Buffer P** and vortex the sample for 5 seconds. Incubate at room temperature for 5 minutes and centrifuge at maximum speed for 5 minutes.
3. Transfer the clear supernatant onto a Prefilter located in a Receiver Tube and centrifuge the tube at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Prefilter.

   Don't discard the Receiver Tube with the filtrate!

NOTE
If there is a pellet after centrifugation, don't discard the pellet. Transfer the supernatant carefully into a new reaction tube.

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

4. Add **200 µl Binding Solution SBS** to the lysed sample (filtrate from step 3), mix by pipetting up and down several times.

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

5. Apply the sample to a Spin Filter located in a new 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. For higher sample volumes repeat the loading step.

6. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
7. Open the Spin Filter and add **650 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. Open the Spin Filter and add **650 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.
9. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
10. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **100–200 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 (e.g. 100 µl + 100 µl) 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 -22 °C to -18 °C is recommended.

11. 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	Select the optimal lysis solutions by side by side preparation of one batch of homogeneously starting material. Increase lysis time. Reduce amount of starting material. Overloading of Spin Filter reduces yield!
Incomplete elution	Prolong the incubation time with **Elution Buffer** to 5 minutes or repeat elution step once again. Take a higher volume of **Elution Buffer** or elute in two steps.
Insufficient mixing with **Binding Solution SBS**	Mix sample with **Binding Solution SBS** by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted DNA	
Too much **Elution Buffer**	Elute the DNA with lower volume of **Elution Buffer**.
Degraded or sheared DNA	
Incorrect storage of starting material	Ensure that the starting material is frozen immediately in liquid N_2 or in minimum at-20 °C and is stored continuously at -80 °C! Avoid thawing of the material.
Old material	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	RNase A digestion

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