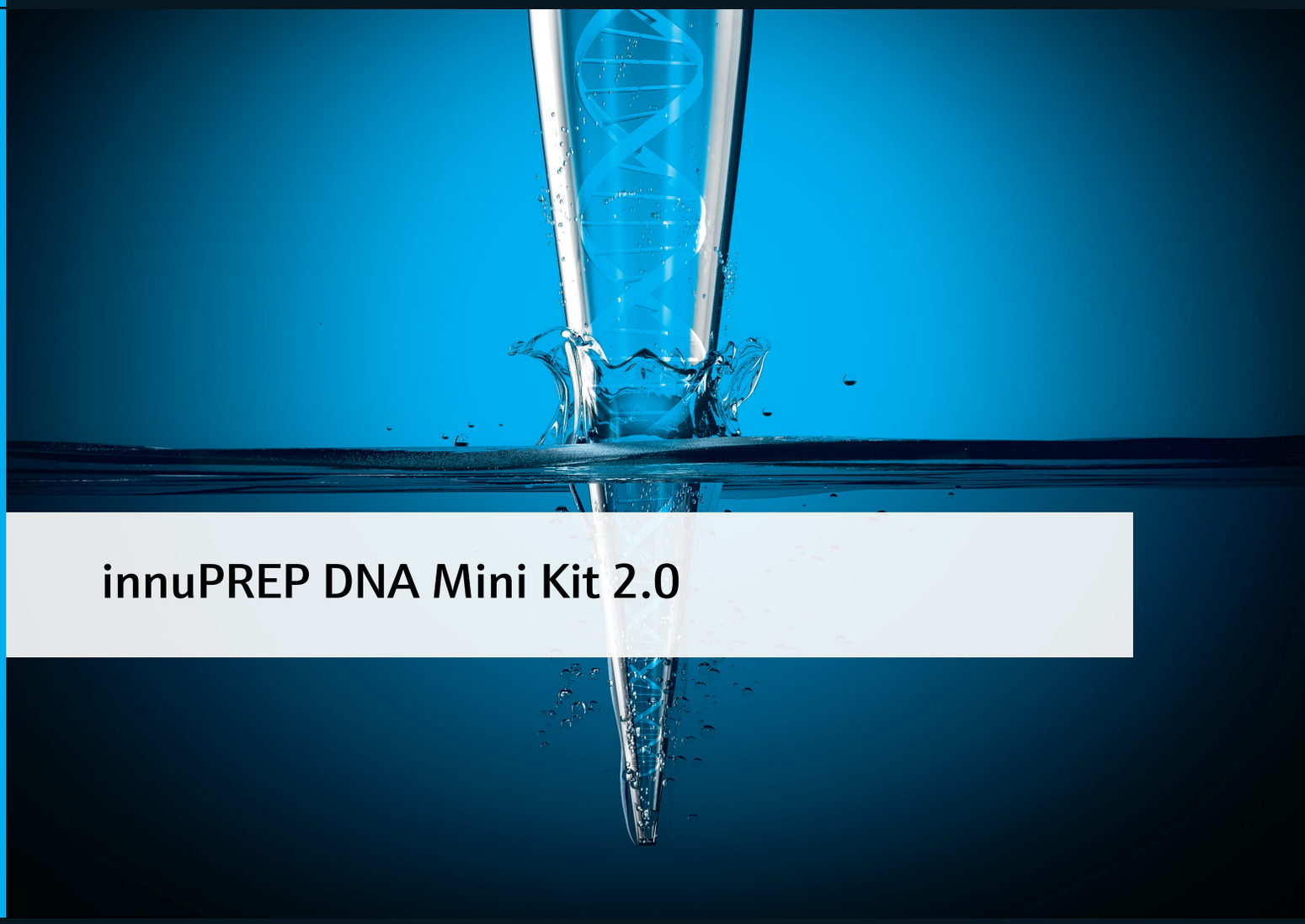
Instructions for Use
Life Science Kits & Assays



innuPREP DNA Mini Kit 2.0

Innuscreen GmbH

Order No.:

845-KS-1042010 10 reactions
845-KS-1042050 50 reactions
845-KS-1042250 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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1 Introduction

1.1 Intended use

The innuPREP DNA Mini Kit 2.0 has been designed as a very efficient tool for fast isolation of genomic DNA from a wide range of starting materials like whole blood (fresh or frozen blood; stabilized with EDTA or citrate, from common blood collection systems), tissue, rodent tails, eucaryotic cells, bacterial cell cultures as well as buccal swabs.

The extraction procedure is based on a new patented chemistry and combines sample lysis with subsequent binding of nucleic acids onto the surface of a Spin Filter membrane. After several washing steps the nucleic acids are eluted from the membrane by using elution buffer. Extraction chemistry and extraction protocol are optimized to get maximum of yield.

The kit is intended for use by professional users. The kit has been designed to be used for a wide range of different downstream applications, like amplification reactions and further analytical procedures.

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 and the kit" p. 4).
- 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 personal in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles 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.innu@ist-ag.com.

3 Storage conditions

The kit is shipped at ambient temperature.

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

Store RNase A at -22 °C to -18 °C.

All other components of the innuPREP DNA Mini Kit 2.0 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 DNA Mini Kit 2.0 or other products, please do not hesitate to contact us. For technical support or further information

in Germany please contact info.innu@ist-ag.com. 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. 10).). 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 equivalent regulations required 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 Components included in the kit

	Σ 10	Σ 50	Σ 250
REF	845-KS-1042010	845-KS-1042050	845-KS-1042250
Lysis Solution CBV	5 ml	25 ml	120 ml
Lysis Solution SLS	5 ml	25 ml	120 ml
Binding Solution BL	8 ml	40 ml	200 ml
Binding Solution SBS	2 ml	12 ml	60 ml
Proteinase K	for 2 x 0.3 ml working solution	for 2 x 1.5 ml working solution	for 6 x 1.5 ml working solution
RNase A (10 mg/ml)	60 µl	300 µl	2 x 300 µl
Washing Solution C	5 ml	25 ml	120 ml
Washing Solution BS (conc.)	2 ml	8 ml	2 x 18 ml
Washing Solution MS (conc.)	6 ml	30 ml	2 x 66 ml
Elution Buffer	6 ml	25 ml	110 ml
Spin Filter	10	50	5 x 50
Receiver Tubes	20	2 x 50	10 x 50
Elution Tubes	10	50	5 x 50
Manual	1	1	1

6.2 Components not included in the kit

- 1.5 and 2.0 ml reaction tubes
- 96–99.8 % ethanol (molecular biology grade, undenaturated)
- ddH_2O for dissolving Proteinase K
- RNase free water for elution steps
- 1 x PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)
- TE Buffer
- Yeast Digest Buffer (50 mM potassium phospaht, 10 mM DTT, pH 7.5)

7 Product specifications

1. Starting material:

- 200 / 400µl fresh or frozen whole blood samples (stabilizers: EDTA or citrate)
- Cell cultures (max. 5 x 10^6 cells)
- Tissue samples (max. 20 mg)
- Rodent tails (0.2 – 0.8 cm tail)
- Bacterial / Yeast cell cultures (max. 10^{10} cells)
- Buccal swabs

NOTE
Avoid freezing and thawing of starting material.

2. Time for isolation:

- Approximately 10 minutes after each lysis step

3. Typical yield:

- Up to > 100 µg gDNA
- Typical ratio A_{260}:A_{280}: 1.8–2.0
- Typical ratio A_{260}:A_{230}: 1.8–2.3

8 Initial steps before starting

- Heat thermal mixer or water bath at 60 °C or 56°C depends on used protocol
- Add the indicated amount of ddH_2O to **Proteinase K**, mix thoroughly and store as described above.

845-KS-1042010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-1042050 845-KS-1042250	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

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

845-KS-1042010	Add 18 ml ethanol to 2 ml Washing Solution BS (conc.).
845-KS-1042050	Add 72 ml ethanol to 8 ml Washing Solution BS (conc.).
845-KS-1042250	Add 162 ml ethanol to 18 ml Washing Solution BS (conc.).

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

845-KS-1042010	Add 14 ml ethanol to 6 ml Washing Solution MS (conc.).
845-KS-1042050	Add 70 ml ethanol to 30 ml Washing Solution MS (conc.).
845-KS-1042250	Add 154 ml ethanol to 66 ml Washing Solution MS (conc.).

- Centrifugation steps should be carried out at room temperature.
- For Lysis steps 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 3–4 times during the incubation. No shaking will reduce the lysis efficiency.
- Pre-fill the needed amount of **RNase free water or Elution Buffer** into a 2.0 ml reaction tube and incubate at 60 °C until the elution step.

9 Protocol for DNA isolation from blood samples

9.1 Protocol 1: Isolation from 200 µl blood samples

IMPORTANT
If the sample volume is less than 200 µl, add the appropriate volume of PBS.

1. Pipette **200 µl** of **whole blood sample** into a 1.5 ml reaction tube.
2. Add **200 µl Lysis Solution SLS and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 10 seconds and incubate the sample at 60 °C for 10 minutes.

NOTE
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of **Binding Solution BL**, vortex shortly and incubate for 5 minutes at room temperature.

3. Optional: centrifuge the 1.5 ml reaction tube for 10 seconds to remove condensate from the lid of the reaction tube.
4. Open the 1.5 ml reaction tube and add **350 µl Binding Solution BL** to the lysed sample. Mix carefully by pipetting up and down several times (3 – 4 times), apply the sample using the pipette to a Spin Filter located in a Receiver Tube and close the cap of the Spin Filter.

IMPORTANT NOTE
Don't vortex the sample at this step!
It is important that the sample and the Binding Solution BL are mixed by pipetting up and down several times. Vortexing will lead to reduced yield of DNA.

5. Centrifuge at 11,000 x g for 1 minute.

   Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back to the Receiver Tube.

6. Open the Spin Filter and add **400 µl Washing Solution C**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back to the Receiver Tube.

7. Open the Spin Filter and add **600 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g for 1 minute.

   Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

8. Open the Spin Filter and add **600 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g for 1 minute.

   Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

9. Centrifuge at max. speed for 3 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 **200 µl RNase free water or Elution Buffer** (pre-warmed at 60 °C). Incubate at room temperature for 2 minutes.

11. Centrifuge at 11,000 x g for 1 minute. Two elution steps with equal volumes of pre-warmed **RNase free water or Elution Buffer** (e.g. 100 µl + 100 µl) might increase the yield of extracted gDNA.

NOTE

The DNA can be eluted with a lower or a higher volume of **RNase free water or Elution Buffer** (depends on the expected yield of genomic DNA). Elution with lower volumes 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.

9.2 Protocol 2: Isolation from 400 µl blood samples

IMPORTANT
If the sample volume is less than 400 µl, add the appropriate volume of PBS.

1. Pipette **400 µl** of **whole blood sample** into a 2.0 ml reaction tube.
2. Add **400 µl Lysis Solution SLS and 30 µl Proteinase K**, mix vigorously by pulsed vortexing for 10 seconds and incubate the sample at 60 °C for 10 minutes.

NOTE
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of **Binding Solution BL**, vortex shortly and incubate for 5 minutes at room temperature.

3. Optional: centrifuge the 2.0 ml reaction tube for 10 seconds to remove condensate from the lid of the reaction tube.
4. Open the 2.0 ml reaction tube and add **700 µl Binding Solution BL** to the lysed sample. Mix carefully by pipetting up and down several times (3 – 4 times), apply **750 µl** of the **sample** using the pipette to a Spin Filter located in a Receiver Tube and close the cap of the Spin Filter.

IMPORTANT NOTE
Don't vortex the sample at this step!
It is important that the sample and the Binding Solution BL are mixed by pipetting up and down several times. Vortexing will lead to reduced yield of DNA.

5. Centrifuge at 11,000 x g for 1 minute.

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

6. Apply the residual sample to the Spin Filter. Close the cap and centrifuge at 11,000 x g for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back to the Receiver Tube.

7. Open the Spin Filter and add **400 µl Washing Solution C**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back to the Receiver Tube.

8. Open the Spin Filter and add **600 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g for 1 minute.

   Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back to the Receiver Tube.

9. Open the Spin Filter and add **600 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g for 1 minute.

   Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back to the Receiver Tube.

10. Centrifuge at max. speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.

11. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **200 µl RNase free water or Elution Buffer** (pre-warmed at 60 °C). Incubate at room temperature for 2 minutes.

12. Centrifuge at 11,000 x g for 1 minute. Two elution steps with equal volumes of pre-warmed **RNase free water or Elution Buffer** (e.g. 100 µl + 100 µl) might increase the yield of extracted gDNA.

NOTE

The DNA can be eluted with a lower or a higher volume of **RNase free water or Elution Buffer** (depends on the expected yield of genomic DNA). Elution with lower volumes 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 Protocol for DNA isolation from tissue samples or rodent tails

1. Cut max. 20 mg of tissue sample or rodent tails into small pieces and place the tissue in a 1.5 ml or 2.0 ml reaction tube. Add **400 µl Lysis Solution CBV** and **30 µl Proteinase K**.

2. Mix vigorously by pulsed vortexing for 5 s. Incubate at 56°C until the sample is completely lysed (appr. 0.5 – 3 h for tissue sample and appr. 2 h for rodent tails).

IMPORTANT
The lysis step should be finished if the material is completely lysed. Optional centrifuge the 1.5 ml reaction tube at maximum speed for 3 minutes to spin down unlysed material. Transfer the supernatant carefully into another 1.5 ml tube.

NOTE
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of **Binding Solution SBS**, vortex shortly and incubate for 5 minutes at room temperature.

3. Add **200 µl Binding Solution SBS** to the lysed sample, mix the sample by pipetting up and down several times. It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

4. Apply the sample to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11,000 x g for 2 minutes.

5. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back to the Receiver Tube.

   Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back to the Receiver Tube.

6. Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back to the Receiver Tube.

7. Open the Spin Filter and add **300 µl Washing Solution MS**, close the cap and centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube and place the Spin Filter into an Elution Tube.

8. Carefully open the cap of the Spin Filter and add **100–400 µl RNase free water or Elution Buffer**. Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g 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 water or Elution Buffer (depends on the expected yield DNA). Elution with lower volumes 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 Protocol for DNA Isolation from cell cultures

1. Pellet cells by centrifugation for 10 min at 5,000 x g. Discard supernatant.

2. Add **100 µl 1 x PBS Buffer** and resuspend the cell pellet completely by pipetting up and down several times.

3. Add **300 µl Lysis Solution CBV** and **25 µl Proteinase K** and mix vigorously by pulsed vortexing for 5 s. Incubate at 56°C until the sample is completely lysed (appr. 15 – 30 minutes depends on number of cells).

NOTE
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of **Binding Solution SBS**, vortex shortly and incubate for 5 minutes at room temperature.

4. Add **200 µl Binding Solution SBS** to the lysed sample, mix by vortexing or by pipetting up and down several times. It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

5. Apply the sample to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11,000 x g for 2 minutes.

6. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

   Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

   Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

7. Open the Spin Filter and add **300 µl Washing Solution MS**, close the cap and centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube and place the Spin Filter into an Elution Tube.

8. Carefully open the cap of the Spin Filter and add **100–400 µl RNase free water or Elution Buffer**. Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g 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 **RNase free water or Elution Buffer** (depends on the expected yield DNA). Elution with lower volumes 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 Protocol for DNA Isolation from bacterial cell cultures

12.1 Collection of bacterial cells

1. Transfer the bacterial culture (volume depends on the concentration of starting material) into a tube 2.0 ml or 1.5 ml tube depends on initial volume.
2. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 minutes at 3,000 x g). Discard the supernatant. Do not discard the pellet!
3. Resuspend the bacterial cell pellet in **100 µl TE-Buffer**.

12.2 Enzymatic lysis

12.2.1 Gram-negative bacteria

NOTE
Although Gram-negative bacteria do not require a pre-lysis step, using Lysozyme (not included in the kit) can enhance the efficiency of lysis.

1. Add **20 µl Lysozyme** (10mg/ml, 400 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
2. Proceed with “Proteolytic lysis step” on p.22.

12.2.2 Gram-positive bacteria

NOTE
Gram-positive bacteria require a pre-lysis step using Mutanolysin and/or Lysozyme (not included in the kit).

1. Add **20 µl Lysozyme** (10mg/ml, 400 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
2. Add **5 µl Mutanolysin** (0.4 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.

NOTE

Lysozyme and Mutanolysin exert synergistic activity. Using both enzymes together will increase the yield of isolated nucleic acids.

3. Proceed with “Proteolytic lysis step” on p.22.

12.2.3 Staphylococcus strains

NOTE

For pre-lysis of *Staphylococcus* the enzyme Lysostaphin is recommended (not included in the kit).

1. Add 1**0 µl Lysostaphin** (0.4 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
2. Proceed with “Proteolytic lysis” on p.22.

12.2.4 Alternative pre-lysis using innuPREP Bacteria Lysis Booster

NOTE

The innuPREP Bacteria Lysis Booster Kit has been developed for a highly efficient pre-lysis of bacterial cell walls by generating spheroblasts. This new mixture of different enzymes boosts the lysis of all bacteria in particular the hard-to-lyse microorganisms like *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Bacillus* and *Clostridium spp*.

1. Prepare the enzyme mix according to the manual of the innuPREP Bacteria Lysis Booster.
2. Add **20 µl enzyme mix** to the sample and vortex shortly. Incubate the sample for 30 minutes at 37 °C under continuous shaking.
3. Proceed with “Proteolytic lysis” on p. 22.

12.3 Proteolytic lysis

1. Add **280 µl Lysis Solution CBV** and **20 µl Proteinase K** to the sample and vortex the sample shortly.
2. Incubate the sample for 30 minutes at 60 °C and 550 rpm in a thermoshaker.
3. Continue with “Bacterial DNA extraction” on p.22.

NOTE
The kit co-purifies DNA and RNA, if DNA and RNA in the sample. If RNA-free genomic DNA is required, add 2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of **Binding Solution SBS**, vortex shortly and incubate for 10 minutes at room temperature.

12.4 Bacterial DNA extraction

1. Add **200 µl Binding Solution SBS** to the lysed sample, mix by pipetting up and down several times.

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

2. Apply the sample to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11,000 x g for 2 minutes.
3. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

   Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

   Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the filtrate

and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

4. Open the Spin Filter and add **300 µl Washing Solution MS**, close the cap and centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube and place the Spin Filter into an Elution Tube.

5. Carefully open the cap of the Spin Filter and add **50–200 µl RNase free water or Elution Buffer**. Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g 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 RNase free water or Elution Buffer (depends on the expected yield of bacterial DNA). Elution with lower volumes 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.

13 Protocol for DNA Isolation from yeast cell cultures

13.1 Collection of yeast cells

1. Pellet cells by centrifugation for 10 min at 5,000 x g. Discard supernatant.
2. Resuspend the yeast cell pellet in **120 µl Yeast Digest Buffer**.

13.2 Enzymatic Lysis

NOTE
For lysis of yeast cells, the enzyme Lyticase is recommended (not included in the kit).

1. Add **10 µl Lyticase** (10 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
2. Proceed with “Proteolytic lysis step”.

13.3 Proteolytic lysis

1. Add **280 µl Lysis Solution CBV** and **20 µl Proteinase K** and vortex the sample shortly.
2. Incubate the sample for 30 minutes at 60°C and 550 rpm in a thermoshaker.
3. Continue with “Yeast DNA extraction”.

NOTE
The kit co-purifies DNA and RNA. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of **Binding Solution SBS**, vortex shortly and incubate for 5 minutes at room temperature.

13.4 Yeast DNA extraction

1. Add **200 µl Binding Solution SBS** to the lysed sample, mix by vortexing or by pipetting up and down several times. It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

2. Apply the sample to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11,000 x g for 2 minutes.

3. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

   Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

   Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

4. Open the Spin Filter and add **300 µl Washing Solution MS**, close the cap and centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube and place the Spin Filter into an Elution Tube.

5. Carefully open the cap of the Spin Filter and add **100–400 µl RNase free water or Elution Buffer**. Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g 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 **RNase free water or Elution Buffer** (depends on the expected yield DNA). Elution with lower volumes 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.

14 Protocol for DNA Isolation from buccal swabs

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

1. Place the swab into a 1.5 ml reaction tube. Add **400 µl Lysis Solution CBV and 25 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds. Incubate at 50 °C for 10–15 minutes under continuous shaking.

2. After lysis time remove the swab from the tube and squeeze the swab on the wall of the tube to remove all **Lysis Solution CBV** from the swab.

NOTE
The kit co-purifies DNA and RNA, if DNA and RNA in the sample. If RNA-free genomic DNA is required, add 2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of **Binding Solution BL**, vortex shortly and incubate for 10 minutes at room temperature.

3. Add **200 µl Binding Solution BL** to the lysed sample, mix the sample by pipetting up and down several times. It is important that the sample and the Binding Solution BL are mixed vigorously to get a homogeneous solution.

4. Apply the sample to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11,000 x g for 2 minutes.

5. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

   Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the filtrate

and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter back into the Receiver Tube.

6. Open the Spin Filter and add **300 µl Washing Solution MS**, close the cap and centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube and place the Spin Filter into a 1.5 ml tube.

7. Carefully open the cap of the Spin Filter and add **100–400 µl RNase free water or Elution Buffer.** Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g 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 RNase free water or Elution Buffer (depends on the expected yield of bacterial DNA). Elution with lower volumes 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.

15 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient disruption or homogenization	Increase lysis time. Increase centrifugation speed. Reduce amount of starting material.
Little or no DNA eluted	
Insufficient lysis	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.
Insufficient mixing with Binding Solution BL or Binding Solution SBS	Mix sample with Binding Solution BL or Binding Solution SBS by pipetting up and down several times prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted DNA	
Too much RNase free H_2O or Elution Buffer	Elute the DNA with lower elution volume.
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 insufficient	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	
RNA contaminations of extracted DNA	Perform RNase digestion

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