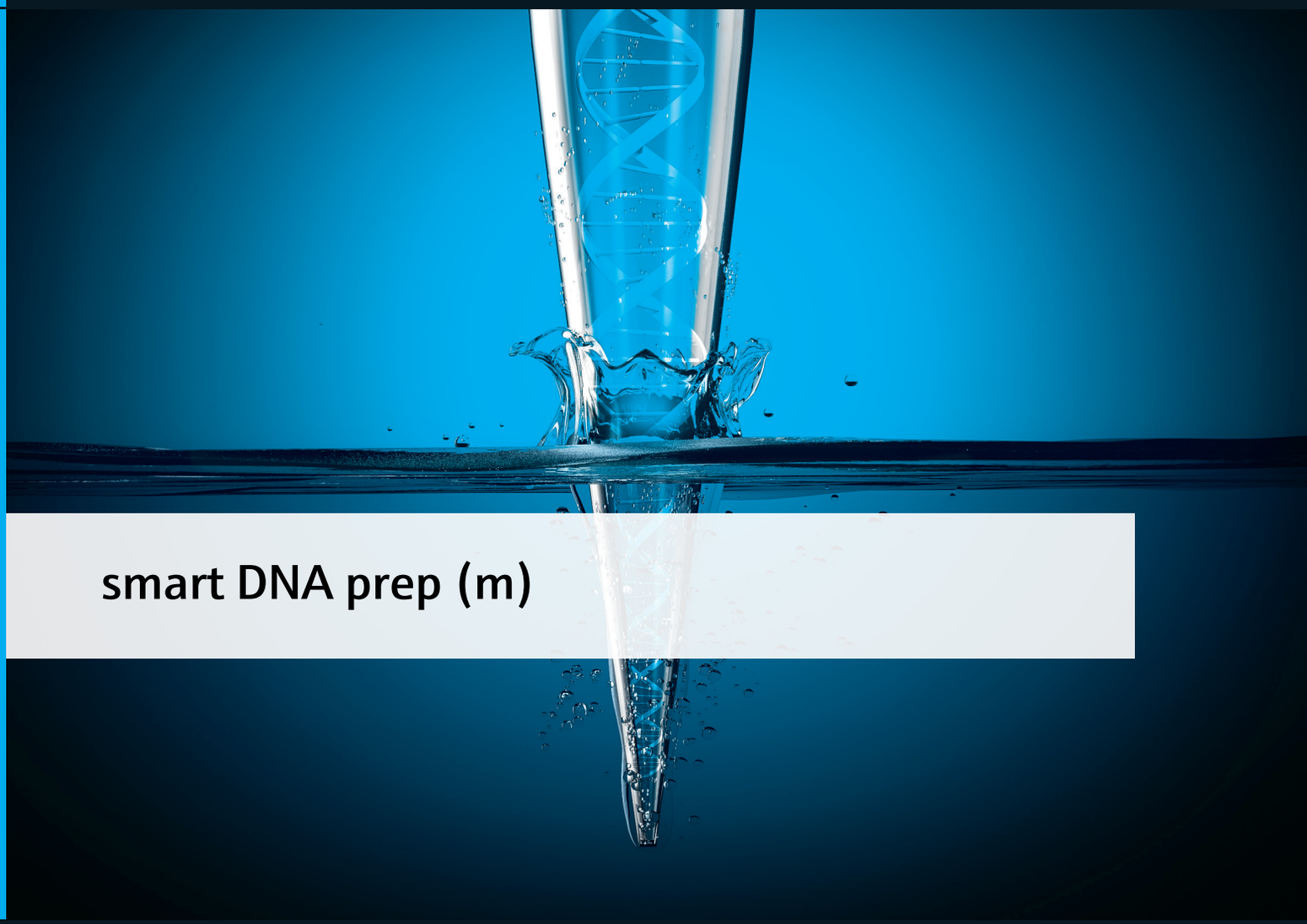
Instructions for Use
Life Science Kits & Assays



smart DNA prep (m)

Innuscreen GmbH

Order No.:

845-KS-8000010 10 reactions

845-KS-8000050 50 reactions

Publication No.: HB_KS-8000_e_220905

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It needs not necessarily agree with future versions. Subject to change!

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Contents

1 Introduction

1.1 Intended use

The **smart DNA prep (m)** kit has been designed for the manual isolation of high molecular weight genomic DNA (200 kb - > 500 kb) from tissue samples, eukaryotic cells, rodent tails, bacteria and yeasts.

The procedure starts with the lysis of the starting material. After lysis the sample is transferred into a SmartExtraction Tube (SE Tube). The SE Tube contains a material with unique Smart Modified Surfaces which adsorbs the genomic DNA. After washing steps, the nucleic acid is dissolved from the surface of the modified material and is now ready to use for downstream applications. The whole extraction process is simple to handle. The unique extraction chemistry in combination with Smart Modified Surfaces is optimized to get a maximum of yield and quality.

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> tests.
15 °C 30 °C	**Storage conditions** Store at room temperature, unless otherwise specified.
	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 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 is designed to be handled only 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 is to be used with potential infectious human 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 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 and dissolved **Proteinase K** at 4 °C to 8 °C.

All other components of the smart DNA prep (m) 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.

If there are any precipitates within the provided solutions dissolve these precipitates by careful warming. Before every use make sure that all components have room temperature.

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 smart DNA prep (m) kit or other IST Innuscreen GmbH 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. 9). 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 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
REF	845-KS-8000010	845-KS-8000050
SE Tube	10	50
Lysis Solution CBV	5 ml	30 ml
Proteinase K	For 2 x 0.3 ml working solution	For 2 x 1.5 ml working solution
Binding Optimizer	1 ml	3 x 1 ml
Washing Solution LS (conc.)	2 ml	12 ml
Elution Buffer	15 ml	2 x 30 ml
Manual	1	1

6.2 Components not included in the kit

- 1.5 ml and 2.0 ml tubes
- 96–98.8 % ethanol (molecular biology grade, undenaturated)
- 80 % ethanol (molecular biology grade, undenaturated)
- 2-Propanol (molecular biology grade)
- ddH_2O for dissolving **Proteinase K**
- optional RNase A (10 mg/ml)
- magnetic rack (Analytik Jena AG, 845-MR-0600001)
- 1 x PBS Buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)

6.3 Components needed for isolation of DNA from bacteria

- Lysozyme (stock solution: 10 mg/ml (400 U/µl))
- Mutanolysin (stock solution: 0.4 U/µl)
- Lysostaphin (stock solution: 0.4 U/µl)

- TE-Buffer

Alternatively: innuPREP Bacteria Lysis Booster

(IST Innuscreen GmbH; 845-KA-1000050)

6.4 Components needed for isolation of DNA from yeasts

- Yeast Digest Buffer (50 mM potassium phosphate, 10 mM DTT, pH 7.5)
- Lyticase (stock solution: 10 U/µl)

7 Initial steps before starting

- Add the indicated volume of ddH_2O to each vial of **Proteinase K**, mix thoroughly and store as described above.

845-KS-8000010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-8000050	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated volume of absolute ethanol to each bottle of **Washing Solution LS (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-KS-8000010	Add 8 ml ethanol to 2 ml Washing Solution LS (conc.).
845-KS-8000050	Add 48 ml ethanol to 12 ml Washing Solution LS (conc.).

8 Product specifications

Starting material:

- Eukaryotic cells (1×10^5–1×10^7)
- Tissue samples (1 mg–100 mg)
- Rodent tail (0.1 cm–1 cm)
- Bacteria cell pellets (1×10^5–1×10^9 cells)
- Yeast cell pellets (1×10^5–1×10^9 cells)

9 Sample preparation for eukaryotic cells

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 5 minutes at 2,500 x g) and discard the supernatant.
2. Add **150 µl PBS** to the cell pellet and resuspend the pellet as much as possible by intensive pipetting up and down.
3. Transfer the resuspended cells into the **SE Tube**.
4. Add **200 µl Lysis Solution CBV** and **40 µl Proteinase K**. Vortex shortly and incubate the **SE Tube** at 55 °C for 20 minutes in a thermal shaker continuously shaking with 1,200 rpm.

   Lysis time of 20 minutes is often sufficient to get enough DNA, but the prolongation of lysis time to 1 hour is also possible.

NOTE
To remove RNA from the sample (optional) add 1 µl of RNase A solution (10 mg/ml), vortex shortly and incubate for 10 minutes at room temperature. Be sure, that the RNase A is free of DNase-activity.

5. Proceed with “SmartExtraction protocol” on p. 15.

10 Sample preparation of tissue samples

10.1 Proteolytic lysis of starting material

1. Cut the starting material into small pieces and put it into a 1.5 ml reaction tube.
2. Add 400 µl Lysis Solution CBV and 40 µl Proteinase K. Vortex shortly and incubate the tube at 55 °C for 1 hour in a thermal shaker continuously shaking with 1,200 rpm. Lysis time of 1 hour is often sufficient to get enough DNA, but the prolongation of lysis time to 3 hours is also possible.
3. After lysis centrifuge the tube at maximum speed for 5 minutes to pellet unlysed material. Carefully transfer the supernatant into the SE Tube.

NOTE
To remove RNA from the sample (optional) add 1 µl of RNase A solution (10 mg/ml), vortex shortly and incubate for 10 minutes at room temperature. Be sure, that the RNase A is free of DNase-activity.

4. Proceed with “SmartExtraction protocol” on p. 15.

Sample preparation of bacteria cell pellets

10.2 Resuspension of starting material

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 minutes at 3,000 x g) and discard the supernatant.
2. Resuspend the bacteria cell pellet in 170 µl TE Buffer. After resuspension start enzymatic pre-lysis as described below. Requirements for pre-lysis depend on the cell type.

10.3 Pre-lysis of resuspended starting material

10.3.1 Gram-negative bacteria

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

Using Lysozyme (stock solution: 10 mg/ml (400 U/µl))

Add **20 µl Lysozyme** to the resuspended cells and incubate at 37 °C for 30 minutes under continuous shaking.

Proceed with “Proteolytic lysis step” on p. 13.

10.3.2 Gram-positive bacteria

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

Using Lysozyme (stock solution: 10 mg/ml (400 U/µl))

Add **20 µl Lysozyme** to the resuspended cells and incubate at 37 °C for 30 minutes under continuously shaking.

Using Mutanolysin (stock solution: 0.4 U/µl)

Add **5 µl Mutanolysin** to the resuspended cells and incubate at 37 °C for 30 minutes under continuously shaking.

Proceed with “Proteolytic lysis step” on p. 13.

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

Alternatively:

Use the **innuPREP Bacteria Lysis Booster**

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*.

Prepare the enzyme mix according to the manual of the innuPREP Bacteria Lysis Booster.

Add **20 µl** of the prepared **enzyme mix** to the sample and vortex it shortly. Incubate the sample for 30 minutes at 37 °C.

Proceed with “Proteolytic lysis step” on p. 13.

10.3.3 Staphylococcus

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

Using Lysostaphin (stock solution : 0.4 U/µl)

Add **10 µl Lysostaphin** to the resuspended cells and incubate at 37 °C for 30 minutes under continuously shaking.

Proceed with “Proteolytic lysis step” on p. 13.

Alternatively:

Use the **innuPREP Bacteria Lysis Booster**

The innuPREP Bacteria Lysis Booster Kit has been developed for a highly efficient pre-lysis of bacterial cell walls by generating sphaeroblasts. 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*.

Prepare the enzyme mix according to the manual of the innuPREP Bacteria Lysis Booster.

Add **20 µl** of the prepared enzyme mix to the sample and vortex it shortly. Incubate the sample for 30 minutes at 37°C.

Proceed with “Proteolytic lysis step” on p. 13.

10.4 Proteolytic lysis step

Transfer the pre-lysed cells into the **SE Tube** and add **200 µl Lysis Solution CBV** and **30 µl Proteinase K**. Vortex shortly and incubate the SE Tube at 55 °C for 30 minutes in a thermal shaker continuously shaking with 1,200 rpm. Lysis time of 30 minutes is often sufficient to get enough DNA. If the sample is not clear after 30 minutes prolong the incubation time until the sample is clear.

NOTE
To remove RNA from the sample (optional) add 1 µl of RNase A solution (10 mg/ml), vortex shortly and incubate for 10 minutes at room temperature. Be sure, that the RNase A is free of DNase-activity.

11 Sample preparation of yeast cell pellets

11.1 Resuspension of starting material

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 minutes with 3,000 x g) and discard the supernatant.
2. Resuspend the yeast cell pellet in 200 µl Yeast Digest Buffer (→"Components needed for isolation of nucleic acids from yeasts" p. 9). After resuspension start enzymatic pre-lysis as described below.

11.2 Pre-lysis of resuspended starting material

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

Using Lyticase (stock solution: 10 U/µl)

Add **10 µl Lyticase** (not included in the kit) to the resuspended cells and incubate at 37 °C for 30 minutes under continuous shaking.

Proceed with "Proteolytic lysis step" on p. 15.

11.3 Proteolytic lysis step

Transfer the pre-lysed cells into the **SE Tube** and add **200 µl Lysis Solution CBV** and **30 µl Proteinase K**. Vortex shortly and incubate the **SE Tube** at 55 °C for 30 minutes in a thermal shaker continuously shaking with 1,200 rpm.

Lysis time of 30 minutes often is sufficient to get enough DNA. If the sample is not clear after 30 minutes prolong the incubation time until the sample is clear.

NOTE
To remove RNA from the sample (optional) add 1 µl of RNase A solution (10 mg/ml), vortex shortly and incubate for 10 minutes at room temperature. Be sure, that the RNase A is free of DNase-activity.

12 SmartExtraction protocol

NOTE
If not otherwise stated, we recommend using a magnetic rack to retain the SE Macro Beads in the SE Tubes and remove supernatants by pouring. Using pipets to aspirate the liquid phase might result in a loss of DNA.

12.1 Binding DNA to SE Macro Beads

1. After lysis add **40 µl Binding Optimizer** and **350 µl 2-Propanol** to the sample in the **SE Tube**.
2. Place the **SE Tube** into a thermal shaker and incubate for 3 minutes at room temperature shaking with 1.400–1.800 rpm.
3. Place the SE Tube into a magnetic rack for separation of the SE Macro Beads. Discard the supernatant.

12.2 Washing and removing of alcohol

1. Add **800 µl of Washing Solution LS** and wash the SE Macro Beads by inverting the **SE Tube** within the magnetic rack five times. Discard the Washing Solution LS (do not remove the SE Tube from the magnetic rack).
2. Add **800 µl of 80 % ethanol** and wash the SE Macro Beads by inverting the **SE Tube** with the magnetic rack five times. Discard the ethanol (do not remove the SE Tube from the magnetic rack).
3. Add **800 µl of 80 % ethanol** and wash the SE Macro Beads by inverting the **SE Tube** within the magnetic rack five times. Discard the ethanol (do not remove the SE Tube from the magnetic rack).
4. Let the **SE Tube** rest in the magnetic rack and wait some seconds. Carefully remove residual ethanol as much as possible using a pipet (also remove ethanol which can be inside the lid).
5. Place the **SE Tube** with opened lid in a thermal shaker and incubate for 15 minutes at 65 °C shaking with 400 rpm to remove the ethanol completely. If the ethanol is not completely removed prolong the incubation time.

12.3 Elution of DNA from SE Macro Beads

1. Add **200 µl–1.000 µl of Elution Buffer** to the **SE Tube**. The amount depends on starting amount of sample and expected yield. Incubate the **SE Tube** in a thermal shaker for 15 minutes at 65 °C shaking with 1.000 rpm. If the DNA is not completely dissolved (DNA is visible as a clump or a strand) a prolongation of the elution step is strongly recommended.
2. Place the **SE Tube** into the magnetic rack and transfer the DNA into a new tube using a pipet.

IMPORTANT NOTE
The extracted DNA is of high molecular weight (200 kb–500 kb). Therefore, the DNA might be very viscous. The dissolving step is crucial for successful extraction and for a maximum of yield. If the DNA content is too high, increase the amount of Elution Buffer and prolong the elution step.
If you do not need high molecular weight DNA you can shear the DNA e.g. by using ultrasound or by passing the eluate through a needle.

13 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
Insufficient lysis	Increase lysis time. Reduce amount of starting material.
Incomplete elution	Prolong the incubation time with Elution Buffer.
Preparation without Binding Optimizer	It is important to add the Binding Optimizer to the lysed sample as described. Binding Optimizer needs to be added after lysis of sample is finished!
High viscosity extracted DNA	
Insufficient amount of Elution Buffer	Elute the DNA with a higher volume of Elution Buffer.
Degraded or sheared DNA	
Old material	Old material often contains degraded DNA.
Pipetting steps performed too rigorously	Pipet more carefully and/or use wide bore pipette tips.

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