

Instructions for Use

Life Science Kits & Assays

A glass pipette tip is shown splashing water. Inside the pipette, a blue DNA double helix is visible. The background is a gradient of blue and black.

innuPREP SE HMW DNA Kit (m)

Order No.:

845-KS-8001010 10 reactions

845-KS-8001050 50 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 SE HMW DNA Kit (m)** kit has been designed for the manual isolation of high molecular weight (HMW) genomic DNA (200 kb – 500+ kb) from tissue samples, eukaryotic cells, rodent tails, bacteria and yeasts based on a patented technology.

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.


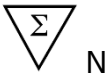
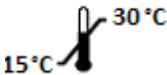





CONSULT INSTRUCTION FOR USE



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 uses the following warning and information symbols as well as the shown methodology:

Symbol	Information
	REF Catalogue number.
	Content Contains sufficient reagents for <N> tests.
	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 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 use 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 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 **innuPREP SE HMW DNA Kit (m)** 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 **innuPREP SE HMW DNA Kit (m)** 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 Components included in the kit

	Σ 10	Σ 50
REF	845-KS-8001010	845-KS-8001050
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
RNase A	60µl	300 µl
Binding Optimizer	1 ml	3 x 1 ml
Washing Solution MS (conc.)	6 ml	30 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)
- 70 % ethanol (molecular biology grade, undenaturated)
- 2-Propanol (molecular biology grade)
- ddH₂O for dissolving **Proteinase K**
- Magnetic Rack (IST Innuscreen GmbH, 845-MR-0600001)
- 1 x PBS Buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄)

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/ μ l)

7 Initial steps before starting

- Add the indicated volume of ddH₂O to each vial of **Proteinase K**, mix thoroughly and store as described above.

845-KS-8001010	Add 0.3 ml ddH ₂ O to lyophilized Proteinase K.
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845-KS-8001050	Add 1.5 ml ddH ₂ O to lyophilized Proteinase K.
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- Add the indicated volume of absolute ethanol to each bottle **Washing Solution MS (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-KS-8001010	Add 14 ml ethanol to 6 ml Washing Solution MS (conc.).
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845-KS-8001050	Add 70 ml ethanol to 30 ml Washing Solution MS (conc.).
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8 Product specifications

Starting material:

- Eukaryotic cells (1 x 10⁵–1 x 10⁷)
- Tissue samples (1 mg–100 mg)
- Rodent tail (0.1 cm–1 cm)
- Bacteria cell pellets (1 x 10⁵–1 x 10⁹ cells)
- Yeast cell pellets (1 x 10⁵–1 x 10⁹ 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 **100 µl PBS** to the cell pellet and resuspend the pellet.
3. Add **280 µl Lysis Solution CBV** and **30 µl Proteinase K**. Vortex briefly and incubate at 55 °C for 20 minutes in a thermal shaker under continuously shaking with 1,000 rpm.

RECOMMENDED

To remove RNA from the sample (optional) add 5 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. Transfer the lysate to the **SE Tube**.
5. Proceed with "SmartExtraction protocol" on p. 15.

10 Sample preparation of tissue samples

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 briefly and incubate the tube at 55 °C for 1 hour in a thermal shaker continuously shaking with 1,000 rpm.
3. After lysis centrifuge the tube at maximum speed for 5 minutes to pellet unlysed material. Carefully transfer the supernatant into the SE Tube.
4. To remove RNA from the sample add **5 µl of RNase A** solution (10 mg/ml), vortex shortly and incubate for 10 minutes.
5. Proceed with "SmartExtraction protocol" on p. 15.

11 Sample preparation of bacteria 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 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.

11.2 Pre-lysis of resuspended starting material

11.2.1 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.

11.2.2 Gram-positive bacteria

Gram-positive bacteria require a pre-lysis-step using Mutanolysin 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.

11.2.3 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.

11.3 Proteolytic lysis step

1. 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 1,000 rpm.
2. To remove RNA from the sample add **5 µl of RNase A solution**, vortex shortly and incubate for 10 minutes at room temperature.

12 Sample preparation of yeast cell pellets

12.1 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.

12.2 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. 14.

12.3 Proteolytic lysis step

3. 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 1,000 rpm.
4. To remove RNA from the sample add **5 µl of RNase A solution**, vortex shortly and incubate for 10 minutes at room temperature.

13 SE 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.

13.1 Binding DNA to SE Macro Beads

1. After lysis add **50 µl Binding Optimizer** and **350 µl 2-Propanol** to the sample in the SE Tube.
 2. Carefully invert the SE Tube 30 times (invert 180°/per cycle 3 seconds). Leave SE tube for 2 min.
-

NOTE

Sample is very viscous at the beginning - viscosity is completely gone in the end.

3. Place the SE Tube into a magnetic rack for separation of the SE Macro Beads. Carefully pour off the supernatant.

13.2 Washing and removing of alcohol

1. Add **800 µl of Washing Solution MS** and wash the SE Macro Beads by inverting the SE Tube within the magnetic rack five times. Carefully pour off the Washing Solution MS (do not remove the SE Tube from the magnetic rack).
2. Add **800 µl of Washing Solution MS** and wash the SE Macro Beads by inverting the SE Tube with the magnetic rack five times. Carefully pour off the Washing Solution MS (do not remove the SE Tube from the magnetic rack).
3. Add **800 µl of 70 % ethanol** and wash the SE Macro Beads by inverting the SE Tube within the magnetic rack five times. Carefully pour off 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 20 minutes at 37 °C shaking with 300 rpm to remove the ethanol completely. If the ethanol is not completely removed prolong the incubation time.

13.3 Elution of DNA from SE Macro Beads

1. Add 150 µl–200 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 30 minutes at 37 °C shaking with 300 rpm. For further analysis please refer to the recommendation of the important note for HMW DNA!!! Let the DNA relax with the beads, don't transfer before relaxing. Let stay the tube with beads over night at 4-8°C.
2. Place the SE Tube into the magnetic rack and transfer the DNA into a new tube using a wide bore pipet tip.

IMPORTANT NOTE HIGH MOLECULAR WEIGHT DNA

The HMW 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/or prolong the elution step. HMW gDNA needs time to relax. It is generally not recommended to work with freshly eluted DNA unless significant effort is made to ensure even DNA resuspension. Letting a sample relax overnight or for several days facilitates homogenization. If possible, it is recommended that HMW DNA is extracted several days or a week prior to being needed for downstream application.

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 or a shredder spin filter unit.

14 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. Refer to important notes!
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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