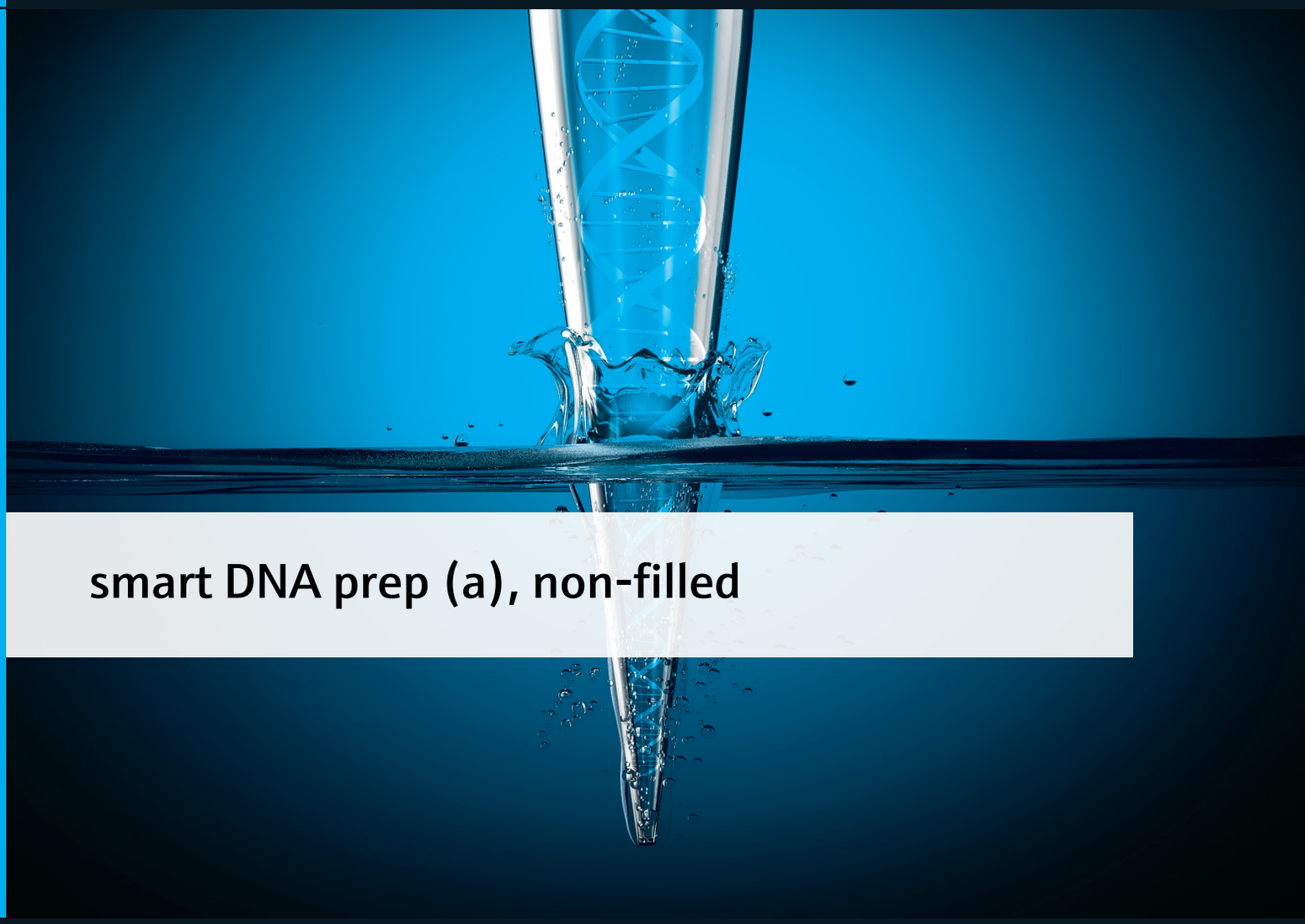
Instructions for Use
Life Science Kits & Assays



smart DNA prep (a), non-filled

iST Innuscreen GmbH

Order No.:

845-ANP-2008016	16 reactions
845-ANP-2008096	96 reactions
845-ANP-2008480	480 reactions

Publication No.: HB_ANP-2008_e_230213

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

Manufacturer and Distributor:
IST Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin · Germany
Made in Germany!

Phone +49 30 9489 3380
Fax +49 30 9489 3381
info.innu@ist-ag.com

Contents

1 Introduction

1.1 Intended use

The **smart DNA prep (a), non-filled** kit has been designed for automated isolation of high molecular weight genomic DNA (gDNA) from tissue samples, cultured eukaryotic cells, rodent tails, bacteria and yeast cells. The kit utilizes the new SmartExtraction technology invented by IST Innuscreen GmbH (patent pending).

The first step of the procedure is the lysis of the starting material. Afterwards, the lysates are transferred into the Reagent Plate of the kit, which must be prefilled with all reagents needed for the extraction process. The extraction is done using a unique 1 ml filter tip in combination with InnuPure C16 / C16 *touch*.

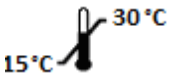
The extraction process is based on adsorption of the genomic DNA to Smart Modified Surfaces inside the tip. After washing, the genomic DNA is eluted from the Smart Modified Surfaces and is ready for subsequent use in downstream applications.

The whole extraction process just needs simple pipetting up and down. The combination of patented, low-salt DC-Technology and patent-pending Smart Modified Surfaces is optimized to get maximum 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** 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 ensure correct function of the device 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 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.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

FOR SINGLE USE ONLY!
This kit is made for single use only!

ATTENTION!
Don’t eat or drink components of the kit!
The kit shall only be handled by educated personnel in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. This kit is to be used with potentially infectious human samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulations.

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 isolation should be free of DNases.

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 the 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 (a), non-filled** 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, they can be dissolved by careful warming.

4 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This product has been produced 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 (a), non-filled** 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 support in other countries, please contact your local distributor.

5 Product use and warranty

The kit is not designed for the usage with other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Intended use”, p. 3) (→ “Product specifications”, p. 10). Since the performance characteristics of IST Innuscreen GmbH kits have only 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 subject 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 16	Σ 96	Σ 480
REF	845-ANP-2008016	845-ANP-2008096	845-ANP-2008480
SmartExtraction Tips	16	96	480
Proteinase K	for 1 x 1.5 ml working solution	for 3 x 1.5 ml working solution	for 13 x 1.5 ml working solution
Lysis Solution CBV	10 ml	2 x 25ml	2 x 120 ml
Deep Well Plate (2.0 ml)	2 (empty)	12 (empty)	60 (empty)
Binding Optimizer	1 ml	5 ml	4 x 5 ml
Washing Solution LS (conc.)	3 ml	15 ml	2 x 36 ml
Elution Buffer	15 ml	70 ml	4 x 80 ml
Buffer ERB	15 ml	70 ml	4 x 80 ml
Filter Tips	1 x 16	1 x 96	5 x 96
Elution Tubes (0.65 ml)	16	2 x 48	10 x 48
Elution Caps (Stripes)	2	12	5 x 12
Manual	1	1	1

6.2 Components not included in the kit

- 1.5 ml and 2.0 ml tubes
- ddH_2O for dissolving **Proteinase K**
- optional RNase A (10 mg/ml)
- 96 %–99.8 % Ethanol (molecular biology grade, undenaturated)

- 1 x PBS Buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)
- 2-Propanol (molecular biology grade)
- 80 % Ethanol (molecular biology grade, undenaturated)

6.3 Related Products

- Deep Well Plate (96 square well, 2.0 ml 845-FX-8500025, 25 pcs)

6.4 Components needed for preparation of 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)

6.5 Components needed for preparation of yeasts

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

7 Initial steps before starting

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

845-ANP-2008016 845-ANP-2008096 845-ANP-2008480	Add **1.5 ml ddH_2O** to lyophilized **Proteinase K.**

- Add the indicated amount of absolute ethanol to **Washing Solution LS** and mix thoroughly. Always keep the bottle firmly closed!

845-ANP-2008016	Add 12 ml of **96-99.8 % ethanol** to 3 ml **Washing Solution LS (conc.)**
845-ANP-2008096	Add 60 ml **96-99.8 % ethanol** to 15 ml **Washing Solution LS (conc.)**
845-ANP-2008480	Add 144 ml **96-99.8 % ethanol** to each bottle 35 ml **Washing Solution LS (conc.)**

8 Product specifications

1. 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)

2. Typical yield:

Not determined. The yield depends on the type and the amount of the starting material

9 Protocols for isolation of DNA

9.1 Protocol 1: Isolation from 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 **200 µl 1 x PBS** to the cell pellet and resuspend the pellet as much as possible by intensive pipetting up and down.
3. Add **200 µl Lysis Solution CBV** and the needed amount of **Proteinase K** (see table below), mix vigorously by pulsed vortexing for 5 seconds.

Number of cells	Proteinase K to be added
1 x 10^5–1 x 10^6	20 µl
1–5 x 10^6	40 µl

4. Incubate at 55 °C for 30 minutes under continuous shaking.

IMPORTANT
Do not use more starting material than described in “Product specifications” on p. 10!

NOTE
We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample every 10 minutes during the incubation. No shaking will reduce the lysis efficiency.

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

5. Proceed with “Preparation of Reagent Plates ” on p. 18.

9.2 Protocol 2: Isolation from tissue samples

1. Cut the starting material into small pieces and place them into a 1.5 ml reaction tube.
2. Add **400 µl Lysis Solution CBV** and **40 µl Proteinase K** and mix vigorously by pulsed vortexing for 5 seconds.
3. Incubate at 55 °C on a shaking platform until the sample is lysed. Sample lysis time depends on amount and kind of sample. Lysis should be completed within 0.5–3 hours.

IMPORTANT
Do not use more starting material than described in "Product specifications" on p. 10!

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

4. After lysis, centrifuge the 1.5 ml tube at 10,000 x g (12,000 rpm) for 2 minutes to spin down unlysed material.
5. Use **400 µl** of the supernatant for the automated extraction.

IMPORTANT
Depending on the sample used, a thin solid phase on the surface of the centrifuged sample can be observed. Avoid contamination of the sample with this solid phase (e.g. fat).

6. Proceed with "Preparation of Reagent Plates " on p. 18.

9.3 Protocol 3: Isolation from bacterial cell pellets

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.

9.3.1 Pre-lysis of resuspended starting material

9.3.1.1 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 of Lysozyme: 10 mg/ml (400 U/µl)

1. Add **20 µl Lysozyme** to the resuspended cells and incubate at 37 °C for 30 minutes under continuous shaking.
2. Proceed with “Proteolytic lysis step” on p. 15.

9.3.1.2 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 of Lysozyme: 10 mg/ml (400 U/µl)

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

Using Mutanolysin: stock solution of Mutanolysin: 0.4 U/µl

1. Add **5 µl Mutanolysin** to the resuspended cells and incubate at 37 °C for 30 minutes under continuous shaking.
2. Proceed with “Proteolytic lysis step” on p. 15.

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, thereby generating spheroplasts. This new mixture of different enzymes boosts the lysis of all bacteria in particular hard-to-lyse microorganisms like *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Bacillus* and *Clostridium*.

1. Prepare the enzyme mix according to the manual of the innuPREP Bacteria Lysis Booster.
2. Add **20 µl** of the prepared **enzyme mix** to the sample and vortex briefly. Incubate the sample for 30 minutes at 37 °C.
3. Proceed with “Proteolytic lysis step” on p. 15.

9.3.1.3 Staphylococcus

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

Using Lysostaphin: stock solution of Lysostaphin: 0.4 U/µl

1. Add **10 µl Lysostaphin** to the resuspended cells and incubate at 37 °C for 30 minutes under continuous shaking.
2. Proceed with “Proteolytic lysis step” on p. 15.

Alternatively: use the innuPREP Bacteria Lysis Booster

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

1. Prepare the enzyme mix according to the manual of the innuPREP Bacteria Lysis Booster.
2. Add **20 µl** of the prepared **enzyme mix** to the sample and vortex it shortly. Incubate the sample for 30 minutes at 37 °C.
3. Proceed with “Proteolytic lysis step” on p. 15.

9.3.2 Proteolytic lysis step

1. Add **200 µl Lysis Solution CBV** and **30 µl Proteinase K** to the sample and mix vigorously by pulsed vortexing for 5 seconds.
2. Incubate sample for 30 minutes at 55°C and 550 rpm on a shaking platform.

   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 briefly and incubate for 10 minutes at room temperature. Ensure that the RNase A is free of DNase-activity.

3. Proceed with “Preparation of Reagent Plates ” on p. 18.

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

3. Proceed with “Preparation of Reagent Plates ” on p. 18.

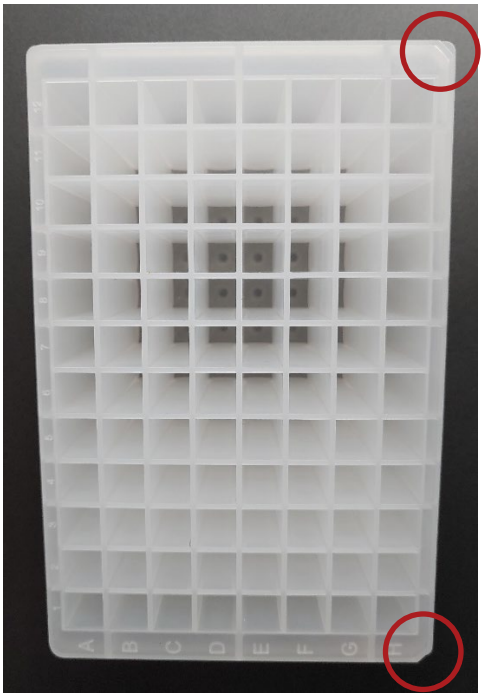
10 Preparation of Reagent Plates

NOTE

The Deep Well Plates have to be filled manually prior to the automated extraction procedure.

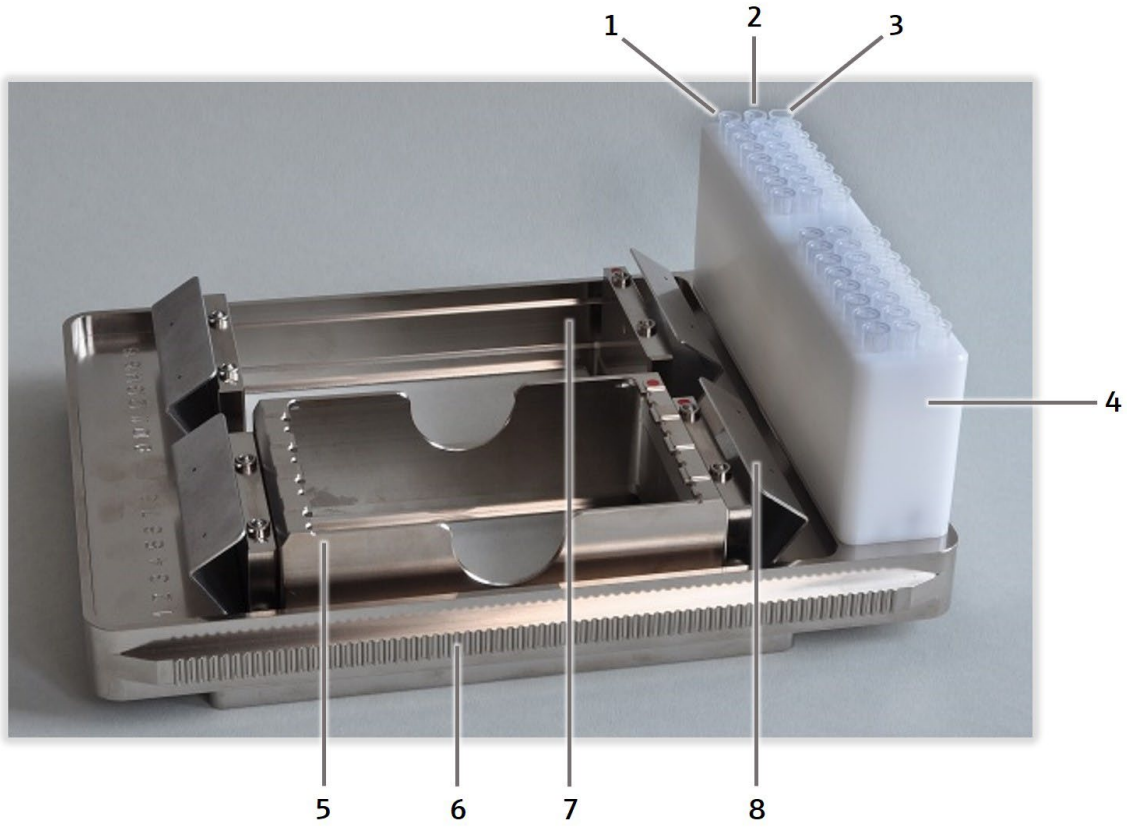
Take care to fill the plates in the correct orientation: Engraved numbers do not coincide with row numbers quoted in the table below!

1. Position the Deep Well Plates in such a way, that the notched corners are facing to the right (see picture below).
2. In this orientation the upper row is row number 1.
3. Fill each cavity of one row with indicated volume of the corresponding solution as specified in the table (e.g. fill each of the eight cavities of row 4 with 600 µl of Isopropanol) and also add **Sample** and **Binding Optimizer** as described in section 11.4 on page 22.

Deep Well Plate	Row No.	Solution	Volume per cavity
	1	empty	---
	2	empty	---
	3	empty	---
	4	Isopropanol	600 µl
	5	Washing Solution LS	600 µl
	6	80% Ethanol	600 µl
	7	80% Ethanol	600 µl
	8	empty	---
	9	Elution Buffer	600 µl
	10	empty	---
	11	Buffer ERB	600 µl
	12	empty	---

11 Automated extraction using InnuPure C16 / C16 touch

11.1 Sample tray of InnuPure C16 / C16 touch



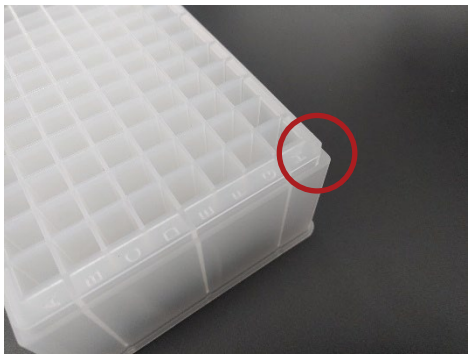
No. 1:	SmartExtraction tips
No. 2:	Standard filter tips
No. 3:	Elution vessels for purified samples
No. 4:	Tip block
No. 5:	Holding-down clamp
No. 6:	Sample block for Reagent Plates or adapter for Reagent Strips
No. 7:	Serrated guide rail (C16 *touch*: non-serrated)
No. 8:	Adapter for Reagent Strips

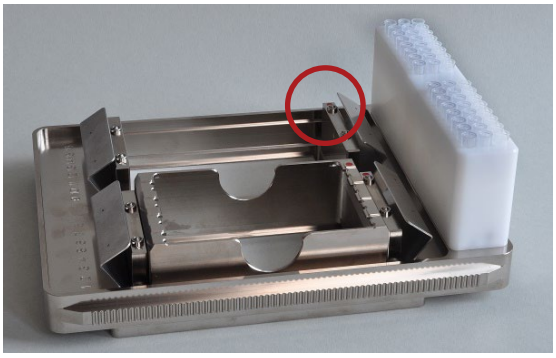
11.2 Preparing the sample tray of InnuPure C16 / C16 touch

4. Place the InnuPure C16 *touch* sample tray into the priming station and open the holding-down clamps of the sample tray!
5. Place the Reagent Plate into the holder of the sample tray. The notched corner of the Reagent Plate has to align with the colored dot on the holder.

Reagent Plate

The notched corner of the Reagent Plate must point to the colored dot on the holder.





CAUTION

Both holders have to be equipped with a Reaction Plate. If applicable, use an empty or dummy plate for the respective holder.

3. Close the holding-down clamp to prevent the Reagent Plates to be pulled out of the holder during the extraction process.
4. For each extracted sample place a SmartExtraction Tip and a filter tip in the smaller drill holes of the tip block (→ "Handling of SmartExtraction Tips" p. 21f.)

NOTE

Extracted high molecular weight DNA from large sample amounts tends to be very viscous. In order to improve the handling of DNA for downstream applications, which do not require high molecular weight DNA, extraction protocols include a homogenization step reducing the fragment size of extracted DNA. If the downstream application requires high molecular weight DNA, no standard filter tips may be put in tip row 2. As a result, the eluate will remain in **cavity 12** of the reagent plastics at the end of the protocol. In this case, transfer of the

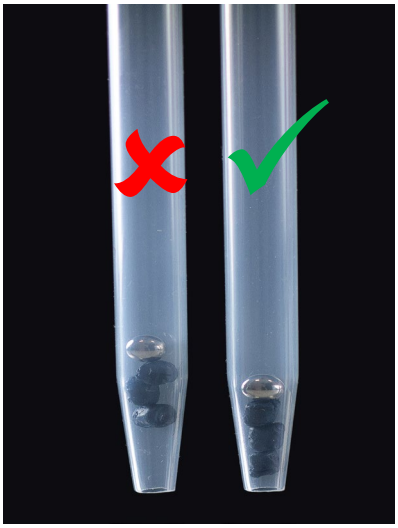
eluate into storage tubes (e.g. Elution Tubes with Elution Caps, 1.5 ml reaction tubes) has to be done manually. In order to avoid loss of DNA integrity pipet carefully with a wide-bore or cut tip.

5. Place the Elution Tubes into the wider drill hole at the edge of the tip block. Empty sample positions do not need to be filled.

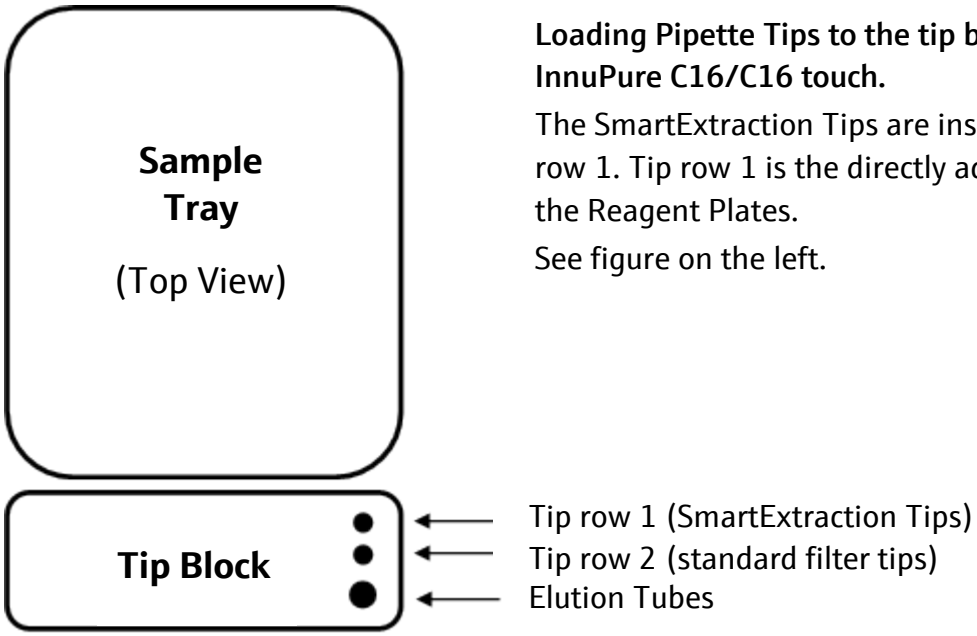
NOTE
Make sure that for every sample the tips and the elution vessel are in the corresponding positions of the tip block!

IMPORTANT NOTE
Use Elution Tubes (0.65 ml) with corresponding Elution Caps.

11.3 Handling of SmartExtraction Tips



Checking the SmartExtraction Tips.
Make sure that the Smart Modified Material is collected near the bottom of the SmartExtraction Tip. If necessary, flick the tip with your finger or against the edge of a table or invert it a few times.
The optimal position of the Smart Modified Material inside the tip is shown in the picture on the left.



Loading Pipette Tips to the tip block of the InnuPure C16/C16 touch.

The SmartExtraction Tips are inserted in tip row 1. Tip row 1 is the directly adjacent to the Reagent Plates.

See figure on the left.

11.4 Loading the sample to InnuPure C16 / C16 touch

1. Prepare the Reagent Plate and the sample tray according to chapter 10 and 11.

NOTE
The following steps will be done after sample lysis!

2. Transfer the whole sample into the **first cavity** (the cavity most distant from the tip block) of the Reagent Plates.
3. Transfer **40 µl** of **Binding Optimizer** to the lysed sample into the **first cavity** of the Reagent Plates.

11.5 Starting the InnuPure C16

1. Switch on the InnuPure C16 and wait for the device initialization to complete, which is signaled by a beeping sound.
2. Move the loaded sample tray with the Reagent Plates forward into the sample tray adapter of the InnuPure C16. The serrated rails at the side of the sample tray must protrude into the grooves of the

adapter. After pressing lightly against the tip block the sample tray is automatically pulled into the device.

IMPORTANT – CAUTION
Risk of injury
Immediately let go of the sample tray once it is being pulled in. Otherwise there is a risk of your hand being injured.

3. After pressing [Select Protocol] choose the appropriate extraction protocol on InnuPure C16 and press [Start]:

Extraction procedure	Protocol on InnuPureC16
Standard (yield optimized, approx. 55 minutes)	SE_Ext_Lysis_C16_01
Fast (time-optimized, approx. 43 minutes)	SE_Ext_Lysis_Fast_C16_01
Sensitive (maximum yield, approx. 67 min)	SE_Ext_Lysis_Sensitive_C16_01

NOTE
For samples with a low or unknown content of DNA always use „Standard protocol" or "Sensitive protocol" for maximum yield. The "Fast protocol" is only recommended for samples with high DNA content in combination with time-critical applications.

4. Enter elution volume and press [OK].

Amount of starting material	Recommended elution volume
< 1 x 10^6 eukaryotic cells	300 µl
1–5 x 10^6 eukaryotic cells	400–500 µl
Bacterial colonies	min. 200 µl
< 1 x 10^8 bacterial cells	min. 200 µl
> 1 x 10^8 bacterial cells	min. 200 µl
< 5 x 10^8 yeast cells	min. 200 µl
> 5 x 10^8 yeast cells	min. 300 µl

5. If needed, choose log file and enter sample IDs, press [OK] or [CANCEL].

NOTE
It is possible to enter sample IDs and to create a run log file. For more detailed information on how to start an extraction protocol using the InnuPure C16 refer to the user manual (“6.3.5 Using the sample setup tool”, p. 37).

6. After completion of the protocol press [NEXT]. The sample tray will be moved out of the device.

NOTE
The chosen protocol is performed by the device and after the protocol is finished, the tray with the purified samples will be moved out of the device upon after pressing [NEXT] and the message 'Program finished' is shown on the screen of the device!

7. Remove the sample tray from the adapter of the InnuPure C16 and move it back into the priming station.
8. After finishing the extraction protocol, the Elution Tubes (0.65 ml) contain the extracted DNA. Close the lids and store the DNA under proper conditions.

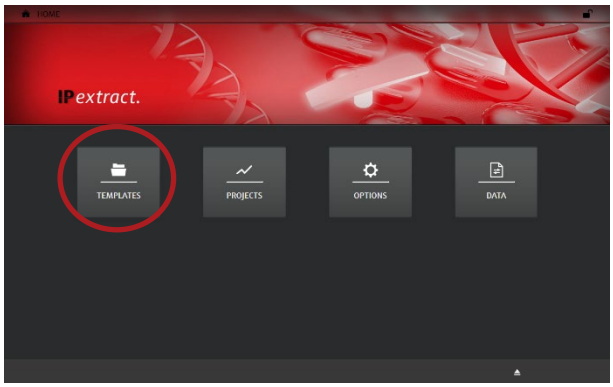
NOTE

Store DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C!

11.6 Starting the InnuPure C16 touch

NOTE
The following instructions describe the necessary steps for the start of the InnuPure C16 *touch*. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure C16 *touch*.

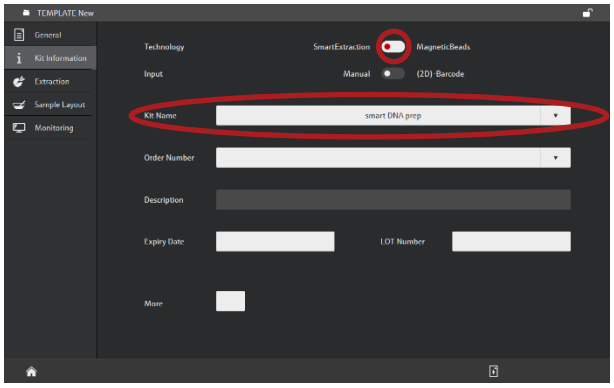
1. Switch on the InnuPure C16 *touch* and the tablet computer. Wait until the home screen of IP*extract* is displayed on the tablet screen.



NOTE
Home screen of IP*extract*

2. Choose [TEMPLATES]→[New Template]→[Kit-based].
3. Enter optional information in the tab “General”.
4. Choose the tab “Kit Information” and switch the “Technology” to “SmartExtraction”!
5. Choose your desired kit from the drop-down list “Kit Name”!

NOTE
“Kit Information” tab

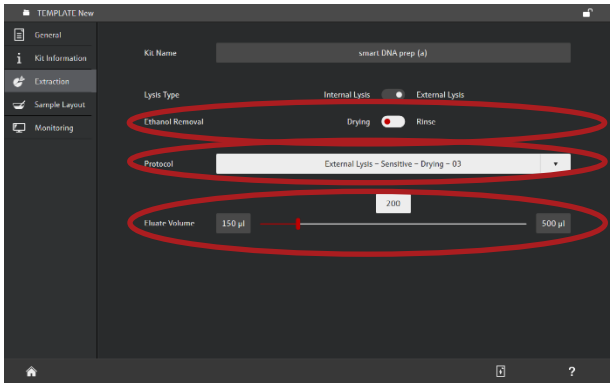


6. Enter optional information in the tab “Kit Information”

7. Choose the tab “Extraction” and choose the desired method for “Ethanol Removal” and “Protocol”

   “Drying” – Ethanol is removed by evaporation

   “Rinse” – Ethanol is washed away using a special Washing Solution



NOTE
“Extraction” tab

Ethanol Removal	Protocol on InnuPureC16 touch
Drying	External Lysis – Drying – 03 (32 minutes) External Lysis – Fast – Drying – 03 (21 minutes) External Lysis – Sensitive – Drying – 03 (67 minutes)
Rinse	External Lysis – Rinse – 03 (28 minutes) External Lysis – Fast - Rinse – 03 (17 minutes) External Lysis – Sensitive – Rinse – 03 (63 minutes)

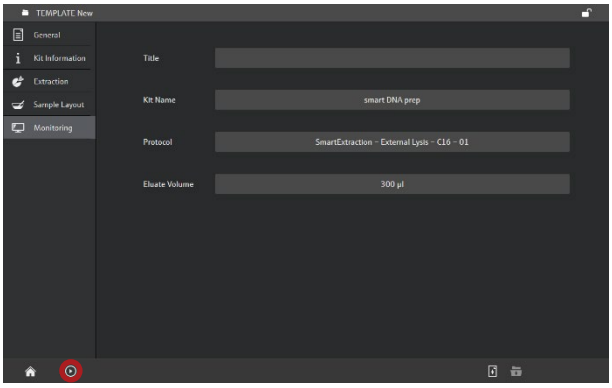
NOTE
For samples with a low or unknown content of DNA always use „Standard protocol" or "Sensitive protocol" for maximum yield. The "Fast protocol" is only recommended for samples with high DNA content in combination with time-critical applications.

NOTE
For most applications, Ethanol Removal by "Drying" is recommended. If the extracted DNA is to be used in very ethanol-sensitive downstream applications (e.g. Droplet PCR), chose the option "Rinse". "Rinse" can also be selected for time-sensitive preparations, since the protocol saves approx. 6 minutes, but the yield might be lower.

8. Adjust your desired "Eluate Volume" using the slider or the text field. Recommended elution volumes are listed in the table below.

Amount of starting material	Recommended elution volume
< 1 x 10^6 eukaryotic cells	300 µl
1–5 x 10^6 eukaryotic cells	400–500 µl
Bacterial colonies	min. 200 µl
< 1 x 10^8 bacterial cells	min. 200 µl
> 1 x 10^8 bacterial cells	min. 200 µl
< 5 x 10^8 yeast cells	min. 200 µl
> 5 x 10^8 yeast cells	min. 300 µl

9. Choose the tab "Monitoring" and start the protocol by tapping the start button.



NOTE
"Monitoring" tab

10. Follow the instructions displayed on the tablet screen.
11. After loading the tray into the device, a message appears reminding you that all cavities must be open before starting. If you have closed the Reagent Plates with a foil, please remove it.

    Please ignore the message if you have not sealed the Reagent Plates. The message must still be confirmed for the protocol start.
12. Completion of the protocol is indicated by a message on the tablet screen. Follow the instructions on the screen to remove the sample tray from the device
13. The Elution Tubes contain the extracted DNA. Close the lids and store the DNA under proper conditions.

NOTE
Store the DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C!

12 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
Insufficient lysis	Increase lysis time. Reduce amount of starting material.
Smart Modified Material not collected near the tip opening	Flick the pipette tip with a finger or against the edge of a table or invert the tip a few times to collect the granules near the bottom of the tip.
Preparation without Binding Optimizer	It is important to add the Binding Optimizer to the Reagent Plastic as described in section 11.4. Pay special attention that Binding Optimizer was added to the lysed sample!
High viscosity of extracted DNA	
Insufficient amount of Elution Buffer	Elute the DNA with a higher volume of Elution Buffer.
Degraded or sheared DNA	
Old sample material	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	RNase A digestion

IST Innuscreen GmbH
Robert-Rössle-Str.10
13125 Berlin · Germany

Phone +49 30 9489 3380
Fax +49 30 9489 3381

info.innu@ist-ag.com

IST Innuscreen GmbH