

Instructions for Use

Life Science Kits & Assays



innuPREP Plant DNA II Kit - IPC16

Order No.:

845-IPS-1616016 16 reactions
845-IPP-1616016 16 reactions
845-IPS-1616096 96 reactions
845-IPP-1616096 96 reactions
845-IPP-1616480 480 reactions

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

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Contents

1	Introduction.....	3
1.1	Intended use.....	3
1.2	Notes on the use of this manual and the kit.....	4
2	Safety precautions.....	5
3	Storage conditions	6
4	Functional testing and technical assistance.....	7
5	Product use and warranty.....	7
6	Kit components	8
6.1	Components included in the kit.....	8
6.2	Components not included in the kit.....	8
7	Initial steps before starting.....	9
8	Product specifications	10
9	Homogenization and lysis of plant samples	11
9.1	Disruption of starting material using a mortar and pestle	11
9.2	Disruption of starting material using bead mill homogenizers	12
9.3	Disruption of starting material using a Rotor-stator homogenizer	12
9.4	Lysis of plant samples	12
9.5	Application example	13
10	Protocol for isolation of genomic DNA (gDNA) from plants	14
10.1	Homogenization of plant material	14
10.2	Protocol 1: gDNA from plant using Lysis Solution SLS	15
10.3	Protocol 2: gDNA from plant using Lysis Solution OPT	16
10.4	Protocol 3: gDNA from plant using Lysis Solution CBV.....	17
11	Preparing Reagent Plate / Strip for automated extraction	18
11.1	General filling scheme of reagent reservoir	18
11.2	Unpacking of Reagent Plate or Reagent Strip.....	18
11.3	Piercing of sealing foil of Reagent Plate or Reagent Strip.....	19

Contents

11.4	Loading the sample to InnuPure C16 <i>touch</i>	20
12	Automated extraction using InnuPure C16 <i>touch</i>	21
12.1	Sample tray of InnuPure C16 <i>touch</i>	21
12.2	Preparing sample tray of InnuPure C16 <i>touch</i>	22
12.3	Starting the InnuPure C16 <i>touch</i>	24
13	Troubleshooting.....	26

1 Introduction

1.1 Intended use

The **innuPREP Plant DNA II Kit - IPC16** has been designed for automated isolation of DNA from plant samples with high content of phenolic ingredients or wood samples using the **InnuPure C16 touch**. The extraction procedure is based on a new-patented chemistry.

The procedure starts with an external lysis step of homogenized plant material followed by the automated extraction of genomic DNA. The kit has been tested for isolation of genomic DNA from leaves, fruits, woods, needles as well as seeds. The starting material can be fresh or frozen. For optimal lysis of plant material the kit contains three different Lysis Solutions. Following lysis the samples are cleared by centrifugation, precipitation and/or filtration using a Prefilter. The samples are then transferred into the Reagent Strip or Reagent Plate of the kit, which is already prefilled with all extraction reagents needed for the extraction process. The following extraction process runs automatically on the **InnuPure C16 touch** and is based on binding of the DNA on surface modified magnetic particles. After washing steps the nucleic acid is eluted and is now ready to use for downstream applications. The extraction chemistry in combination with the **InnuPure C16 touch** protocol are optimized to get 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 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, 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 by educated personnel in a laboratory environment!

If bottles or plates are damaged or leaking, wear gloves and protective goggles when discarding the bottles or plates 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 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 and dissolved **Proteinase K** at 4 °C to 8 °C!

All other components of the **innuPREP Plant DNA II Kit – IPC16** 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 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 Plant DNA II Kit – IPC16** 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. 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 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

	 16	 96	 480
REF	845-IPS-1616016 ^a 845-IPP-1616016 ^b	845-IPS-1616096 ^a 845-IPS-1616096 ^b	845-IPP-1616480 ^b
Lysis Solution SLS	12 ml	60 ml	260 ml
Lysis Solution OPT	10 ml	2 x 25 ml	125 ml
Lysis Solution CBV	10 ml	2 x 25 ml	250 ml
Proteinase K	for 2 x 0.3 ml working solution	for 2 x 1.5 ml working solution	for 7 x 1.5 ml working solution
Precipitation Buffer P	6 ml	2 x 6 ml	2 x 30 ml
Prefilter	16	2 x 50	10 x 50
Receiver Tubes	20	2 x 50	10 x 50
Reagent Strip E ^a	16 (pre-filled, sealed)	96 (pre-filled, sealed)	--
Reagent Plate E ^b	2 (pre-filled, sealed)	12 (pre-filled, sealed)	60 (pre-filled, sealed)
Filter Tips	2 x 16	2 x 96	10 x 96
Elution Tubes (0.65 ml)	16	2 x 48	10 x 48
Elution Caps (Stripes)	2	12	5 x 12
Elution Stripes	2	12	5 x 12
Manual	1	1	1

6.2 Components not included in the kit

- ddH₂O for dissolving Proteinase K
- RNase A (10 mg/ ml), optional
- 1.5 ml tubes
- 2.0 ml tubes, optional
- innuPREP Lysis Tube P (Order-no. 845-CS-1020050, -1020100)

- Piercing Tool (12 well Piercer; 845-PTS-0000005, IST Innuscreen GmbH, Germany)

7 Initial steps before starting

- Invert the Reagent Plate / Reagent Strips for 3–4 times and thump it onto a table to collect the prefilled solutions at the bottom of the wells
- Add the indicated amount of ddH₂O to each vial of **Proteinase K**, mix thoroughly and store as described above.

845-IPS-1616016	Add 0.3 ml ddH ₂ O to lyophilized Proteinase K.
845-IPP-1616016	

845-IPS-1616096	Add 1.5 ml ddH ₂ O to lyophilized Proteinase K.
845-IPP-1616096	
845-IPP-1616480	

- Heat thermal mixer or water bath at 65 °C.

8 Product specifications

1. Starting material:

- Plant samples
- fresh, frozen or dried starting material
- 50 - 100 mg dry weight or
- 120 - 180 mg wet weight

2. Time for isolation:

Preliminary steps: approx. 1 hour

Extraction protocol	Protocol on InnuPure C16 touch	Time InnuPure C16 touch	Elution volumes
External Lysis 200µl – 05	200 µl	52 min	20–500 µl
External Lysis 200µl – Fast – 05	200 µl	41 min	20–500 µl

3. Typical yield:

- depends on type and amount of the starting material
- the amount of DNA that can be expected per mg of sample and depends on the size and ploidy of the genome

9 Homogenization and lysis of plant samples

In case plant samples are not processed immediately after harvesting, it can be:

- kept at 4 °C to 8 °C for 24 hours
- lyophilized/dried within 24 hours of collection and store at 15 °C to 30 °C
- frozen at -18 °C to -22 °C (long time storage at -78 °C to - 82 °C)
- stored in liquid nitrogen

It is recommended to collect young materials (e.g. leaves, needles) since they contain more cells per weight and therefore result in higher yields of DNA extracted. In addition, young leaves and needles contain smaller amounts of polysaccharides and polyphenolics and are therefore easier to process. Complete and quick disruption of starting material is essential to ensure high DNA yields and to avoid DNA degradation. The lysis procedure is most effective with well-homogenized, powdered samples. Suitable methods include any type of commercial homogenizers (rotor-stator homogenizer) or bead mills using ceramic beads. However, we recommend grinding with a mortar and pestle in the presence of liquid nitrogen to obtain optimal yields. When using tissues other than leaves, the disruption method may require optimization to ensure maximum DNA yield and quality. After homogenization and treatment of the sample with lysis solution, the crude lysate can be cleared easily either with Prefilters or by centrifugation.

9.1 Disruption of starting material using a mortar and pestle

Use mortar and pestle to grind the plant sample in the presence of liquid nitrogen to a fine powder. Freeze plant material in liquid nitrogen and be careful during homogenization, because do not let the sample thaw at any time. We recommend precooling the used laboratory equipment and using precooled tubes for sample storage until lysis step. Make sure no liquid nitrogen is transferred or all nitrogen has evaporated before closing the tube.

9.2 Disruption of starting material using bead mill homogenizers

Use 0,5 g ceramic beads (e.g. 2,4 – 2,6 mm ceramic beads – Lysis Tube P, IST Innuscreen GmbH) for plant material and leaves or 4 - 5 steel beads in a mixture (e.g. 4,7 mm diameter steel beads – Lysis Tube Z, IST Innuscreen GmbH) for seeds, rice and needles.

Pipette 50 µl ddH₂O to the plant material and homogenize for about 30 seconds. Repeat the homogenization procedure until the entire plant material is ground to a fine solution.

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

9.3 Disruption of starting material using a Rotor-stator homogenizer

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

9.4 Lysis of plant samples

NOTE

To obtain optimal DNA yield, it might be advantageous to process a higher than the recommended sample mass. However, to ensure a complete lysis, all lysis solution volumes must be increased proportionally.

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

The standard protocol uses **Lysis Solution SLS**, containing CTAB as detergent component. Additionally, the SDS based **Lysis Solution OPT** is provided which requires subsequent precipitation step to remove all impurities by **Precipitation Buffer P**. For some plant species a third **Lysis Solution CBV** leads to higher yield than both other Lysis Solutions. Lysis Solution CBV requires a subsequent precipitation step, too.

NOTE

To find optimal lysis conditions when using a certain plant sample for the first time, it is recommended to do side-by-side preparations of one batch of homogeneously ground material with the three different lysis solutions.

9.5 Application example

The following example of application illustrates the effects of different lysis solutions on yield and quality of the extracted genomic DNA.

Isolation of gDNA from parsley (*Petroselinum crispum*) using the three different lysis solutions SLS, OPT and CBV. The spectrophotometric measurement shows different results depending on the lysis solution used.

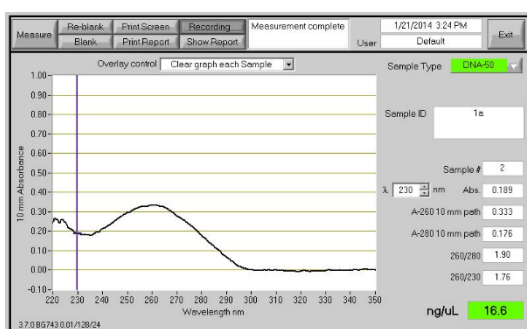


Fig. 1: Lysis Solution SLS
(yield: 16.8 ng/μl; purity (A₂₆₀/A₂₃₀): 1.76)

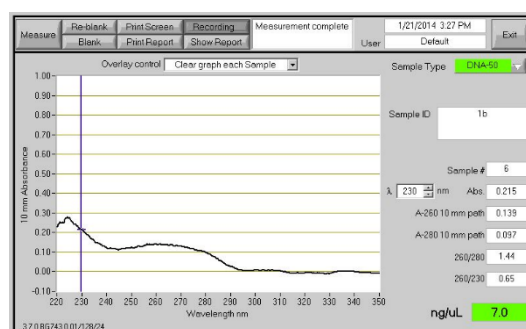


Fig. 2: Lysis Solution OPT
(yield: 7.0 ng/μl; purity (A₂₆₀/A₂₃₀): 0.65)

Protocol for isolation of genomic DNA (gDNA) from plants

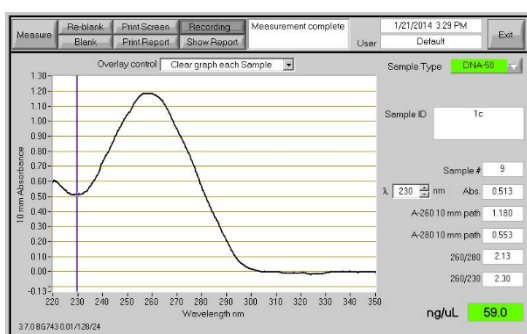


Fig. 3: Lysis Solution CBV

(yield: 59.0 ng/μl; purity (A_{260}/A_{230}): 2.30)

NOTE

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

10 Protocol for isolation of genomic DNA (gDNA) from plants

10.1 Homogenization of plant material

NOTE

The lysis of the starting material is a preliminary manual processing step. To maximize the final yield of DNA a complete homogenization of plant sample is important!

1. Homogenization of about 50 - 100 mg of starting material by:
 - pestle under liquid N_2 . or
 - pestle in present of sand or
 - Homogenizators

NOTE

Use 120–180 mg of starting material if the plant material has a high water. Please note chapter "Homogenization and lysis of plant samples" on p. 11.

2. Choosing protocol for lysis of plant material

Lysis Solution SLS	Protocol 1	(→p. 15)
Lysis Solution OPT	Protocol 2	(→p. 16)
Lysis Solution CBV	Protocol 3	(→p. 17)

NOTE

To find optimal lysis conditions when using a certain plant sample for the first time, it is recommended to do side-by-side preparations of one batch of homogeneously ground material with the three different lysis solutions.

10.2 Protocol 1: gDNA from plant using Lysis Solution SLS

1. Transfer the plant powder or other homogenized starting material in a 1.5 ml or 2.0 ml reaction tube. Add **500 µl Lysis Solution SLS** and **20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds.
 2. Incubate at 65 °C for 30 minutes to 60 minutes.
-

NOTE

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

3. Transfer the sample onto a Prefilter located in a Receiver Tube and centrifuges the tube at 11,000 x g for 1 minute. Discard the Prefilter. The filtrate is used for automated extraction.
-

NOTE

Don't discard the Receiver Tube with the filtrate!
Don't discard the pellet at the bottom of the Receiver Tube.

NOTE

To remove RNA from the sample (if necessary) add 1–2 µl of RNase A solution (10 mg/ml) to the filtrate, vortex shortly and incubate for 5 minutes at room temperature.

4. Proceed with automated extraction (→ "Preparing Reagent Plate / Strip for automated extraction ", p. 18).

10.3 Protocol 2: gDNA from plant using Lysis Solution OPT

1. Transfer the plant powder or other homogenized starting material in a 1.5 ml or 2.0 ml reaction tube. Add **500 µl Lysis Solution OPT**, mix vigorously by pulsed vortexing for 5 seconds.
2. Incubate at 65 °C for 30 minutes to 60 minutes.

NOTE

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

3. Add **100 µl Precipitation Buffer P** and vortex the sample for 5 seconds. Incubate at room temperature for 5 minutes and centrifuge at maximum speed for 5 minutes.
4. Transfer the clear supernatant onto a Prefilter located in a Receiver Tube and centrifuges the tube at 11,000 x g for 1 minute. Discard the Prefilter. The filtrate is used for automated extraction.

NOTE

Don't discard the Receiver Tube with the filtrate!
Don't discard the pellet at the bottom of the Receiver Tube.

NOTE

To remove RNA from the sample (if necessary) add 1–2 µl of RNase A solution (10 mg/ml) to the filtrate, vortex shortly and incubate for 5 minutes at room temperature.

5. Proceed with automated extraction (→ "Preparing Reagent Plate / Strip for automated extraction ", p. 18).

10.4 Protocol 3: gDNA from plant using Lysis Solution CBV

1. Transfer the plant powder or other homogenized starting material in a 1.5 ml or 2.0 ml reaction tube. Add **500 µl Lysis Solution CBV** and **20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds.

NOTE

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

2. Incubate at 65 °C for 30 minutes to 60 minutes).
3. Add 100 µl Precipitation Buffer P and vortex the sample for 5 seconds. Incubate at room temperature for 5 minutes and centrifuge at maximum speed for 5 minutes.
4. Transfer the clear supernatant onto a Prefilter located in a Receiver Tube and centrifuges the tube at 11,000 x g for 1 minute. Discard the Prefilter. The filtrate is used for automated extraction.

NOTE

Don't discard the Receiver Tube with the filtrate!
Don't discard the pellet at the bottom of the Receiver Tube.

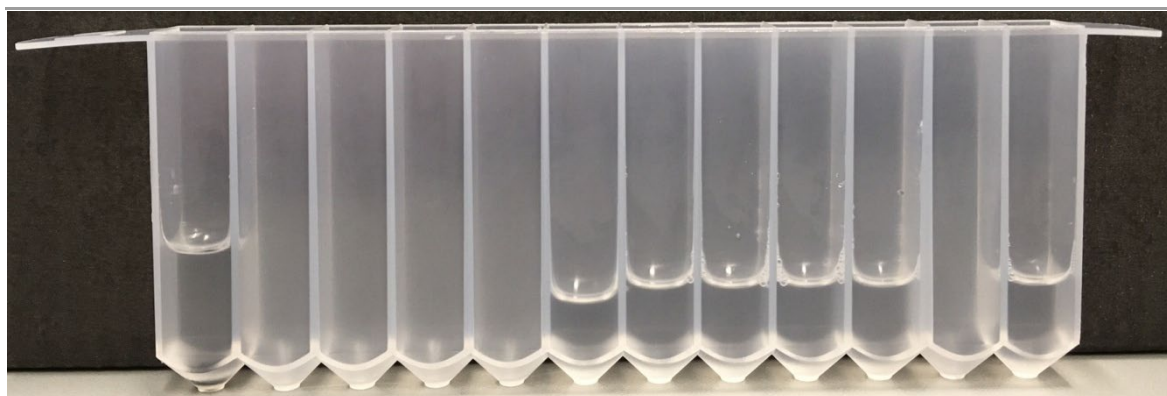
NOTE

To remove RNA from the sample (if necessary) add 1–2 µl of RNase A solution (10 mg/ml) to the filtrate, vortex shortly and incubate for 5 minutes at room temperature.

6. Proceed with automated extraction (→ "Preparing Reagent Plate or Reagent Strip for automated extraction ", p. 18).

11 Preparing Reagent Plate / Strip for automated extraction

11.1 General filling scheme of reagent reservoir



Cavity 1:	Magnetic particles	Cavity 7:	Washing Solution
Cavity 2:	Empty	Cavity 8:	Washing Solution
Cavity 3:	Empty	Cavity 9:	Washing Solution
Cavity 4:	Empty	Cavity 10:	Washing Solution
Cavity 5:	Empty	Cavity 11:	Empty
Cavity 6:	Binding Solution	Cavity 12:	Elution Buffer

11.2 Unpacking of Reagent Plate or Reagent Strip

NOTE

According to transport regulations Reagent Reservoirs are wrapped into plastic bags only when transported by airplane.



Reagent Plates or Reagent Strips are delivered wrapped into plastic bags for transport protection.

Carefully open the overpack of Reagent Plates or Strips by using scissors.

11.3 Piercing of sealing foil of Reagent Plate or Reagent Strip

NOTE

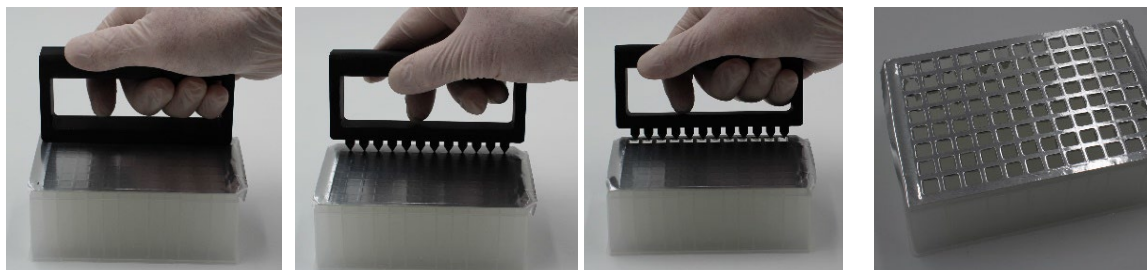
Before using Reagent Plates or Strips the sealing foil has to be pierced manually. Always wear gloves while piercing of the foil!



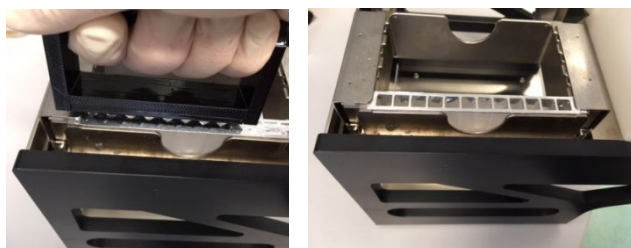
Reagent Plates or Strips are prefilled with extraction reagents and are sealed with a foil. Prior to use this foil has to be pierced manually, by using the piercing tools (12 well piercer).

Keep the Reagent Plates or Strips in a horizontal position to avoid spilling of the reagents while piercing of the foil. Open all cavities (one row per sample).

Using Reagent Plates



Using Reagent Strips



11.4 Loading the sample to InnuPure C16 *touch*

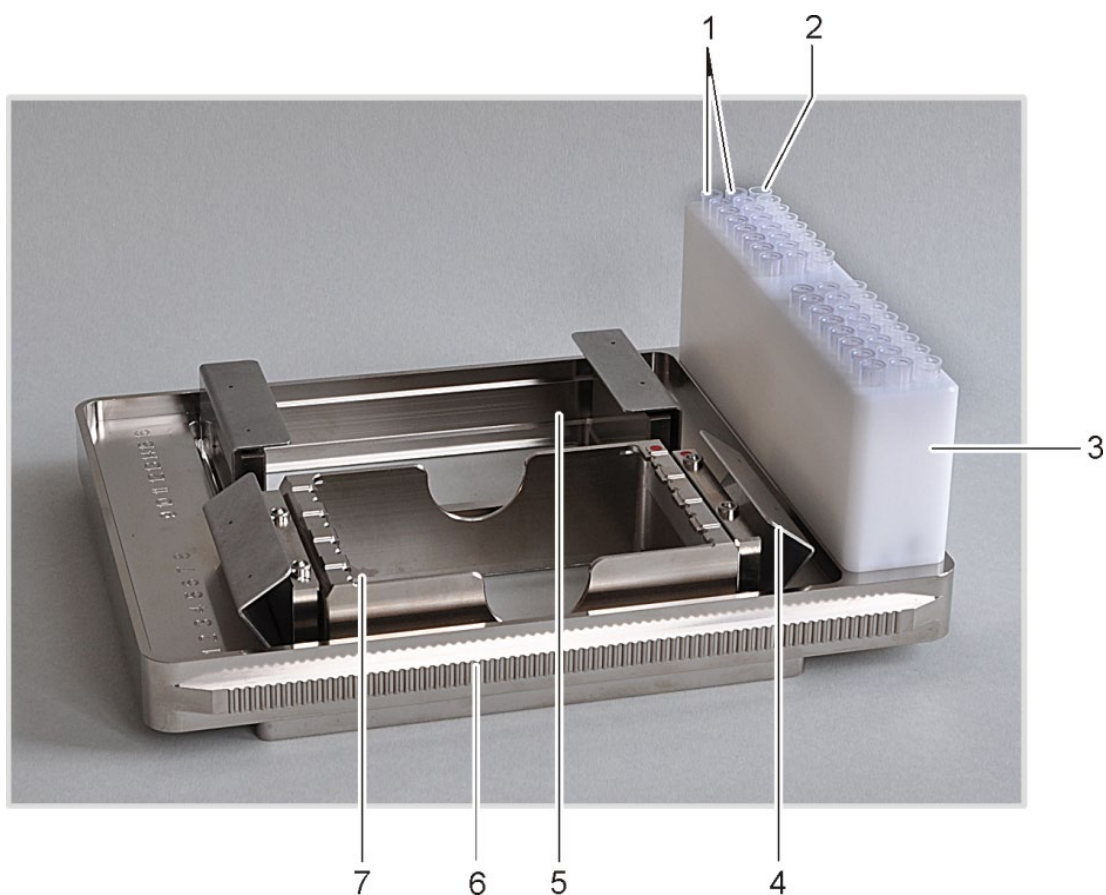
1. Ensure the foils of Reagent Plate or Reagent strips have been pierced (→ „Preparing Reagent Plate / Strip for automated extraction“ p. 18).
2. Transfer **400 µl of lysed sample** into the **third cavity** of Reagent Strip or Reagent Plate. Avoid carry-over of residual solid material!

NOTE

The sample will be processed using the InnuPure C16 *touch*. Please follow the instructions of chapter 12 on p. 21.

12 Automated extraction using InnuPure C16 touch

12.1 Sample tray of InnuPure C16 touch



No. 1: Filter tips

No. 2: Elution vessels for purified samples

No. 3: Tip block

No. 4: Holding-down clamp

No. 5: Sample block for Reagent Plates or adapter for Reagent Strips

No. 6: Guide rail

No. 7: Adapter for Reagent Strips

12.2 Preparing sample tray of InnuPure C16 touch

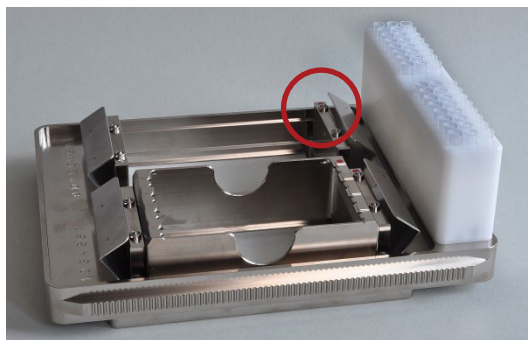
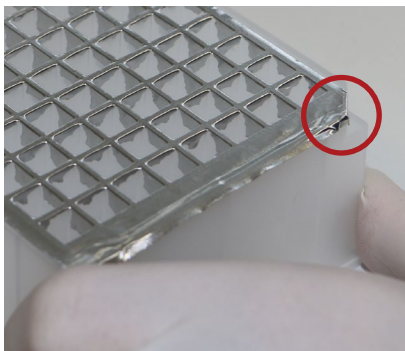
NOTE

The needed number of Reagent Strips or Reagent Plates is depending on the number of samples, which have to be processed. Don't use more strips as number of samples!

1. Place the InnuPure C16 *touch* sample tray into the priming station and fold the holding-down clamp at the sample tray upwards!
2. Place the Reagent Plate or an adapter with Reagent Strips into the holder of the sample tray. Using Reagent Plates, the notched corner of the Reagent Plate has to align with the colored dot at the holder. Using adapters and Reagent Strips, the colored dot of the adapter has to align with the colored dot at the holder and Reagent Strips have to be inserted in a way that the "AJ" labels are arranged at the side of the adapter which is more distant from the tip block.

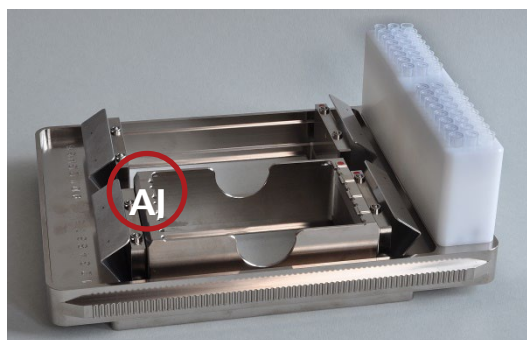
Reagent Plate

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



Reagent Strips

Place the Reagent Strips into the adapter. The long tab marked with the label "AJ" must point to the side of the adapter which is more distant from the tip block.



CAUTION

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

3. Fold down 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 two filter tips in the smaller drill holes of the tip block.
 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

Especially with the Reagent Strips make sure that for every strip the tips and the elution vessel are in the corresponding positions in the tip block!

IMPORTANT NOTE

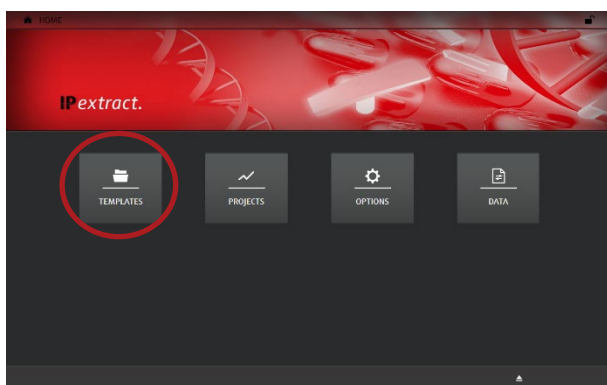
It is possible to select between two different elution vessels! For small elution volumes up to 200 μ l use Elution Strips (0.2 ml). For high elution volumes up to 500 μ l use Elution Tubes (0.65 ml) with corresponding Elution Caps (Strips).

12.3 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.

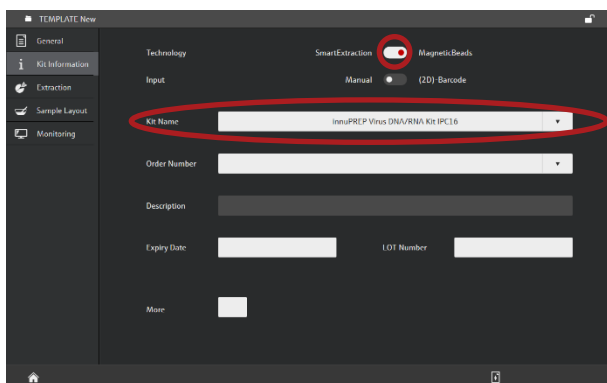
1. Switch on the InnuPure C16 touch and the tablet computer. Wait until the home screen of IPextract is displayed on the tablet screen.



NOTE

Home screen of IPextract

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 "MagneticBeads"!
5. Choose your desired kit from "Kit Name"!



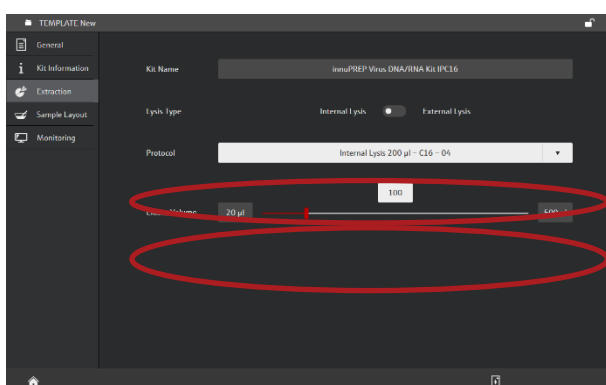
NOTE

"Kit Information" tab

6. Enter optional information in the tab "Kit Information"
7. Choose the tab "Extraction" and choose the desired "Protocol":

Extraction procedure	Protocol on InnuPure C16 <i>touch</i>
Standard (maximum yield, approx. 52 minutes)	External Lysis 200 μ l - 05
Fast (time-optimized, approx. 41 minutes)	External Lysis 200 μ l - Fast - 05

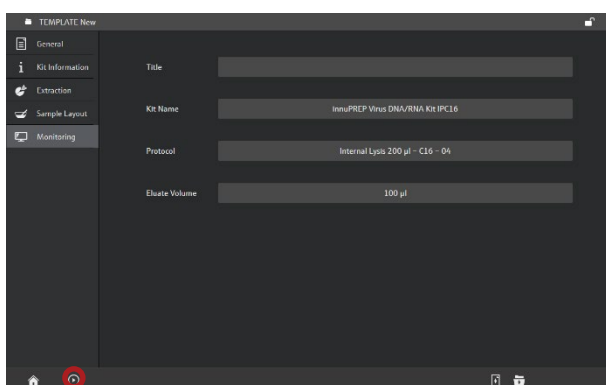
8. Adjust your desired “Eluate Volume” using the slider or the text field.



NOTE
“Extraction” tab

The elution volume is 200 μ l.
Don’t use a higher elution volume.

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



NOTE
“Monitoring” tab

- Follow the instructions displayed on the tablet screen.
- 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.
- The Elution Tubes contain the extracted DNA. Close the lids and store eluate under proper conditions.

NOTE

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

13 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted genomic DNA	
Low yield of extracted DNA	Please try one of the other Lysis Solutions delivered with the kit.
Poor quality of extracted DNA	Avoid carryover of residual plant material when transferring lysed sample to cavity 3 of Reagent Plate/Strip. Please note that Lysis Solution OPT as well as Lysis Solution CBV requires a precipitation step.
Insufficient lysis of starting material	Perform lysis at 65 °C for at least 30 minutes. Ensure to use sufficient Lysis Solution!
Elution volume too high	Decrease the elution volume. The suggested elution volume is 200 µl. Please note that lowering the elution volume will not necessarily increase the yield proportional!

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