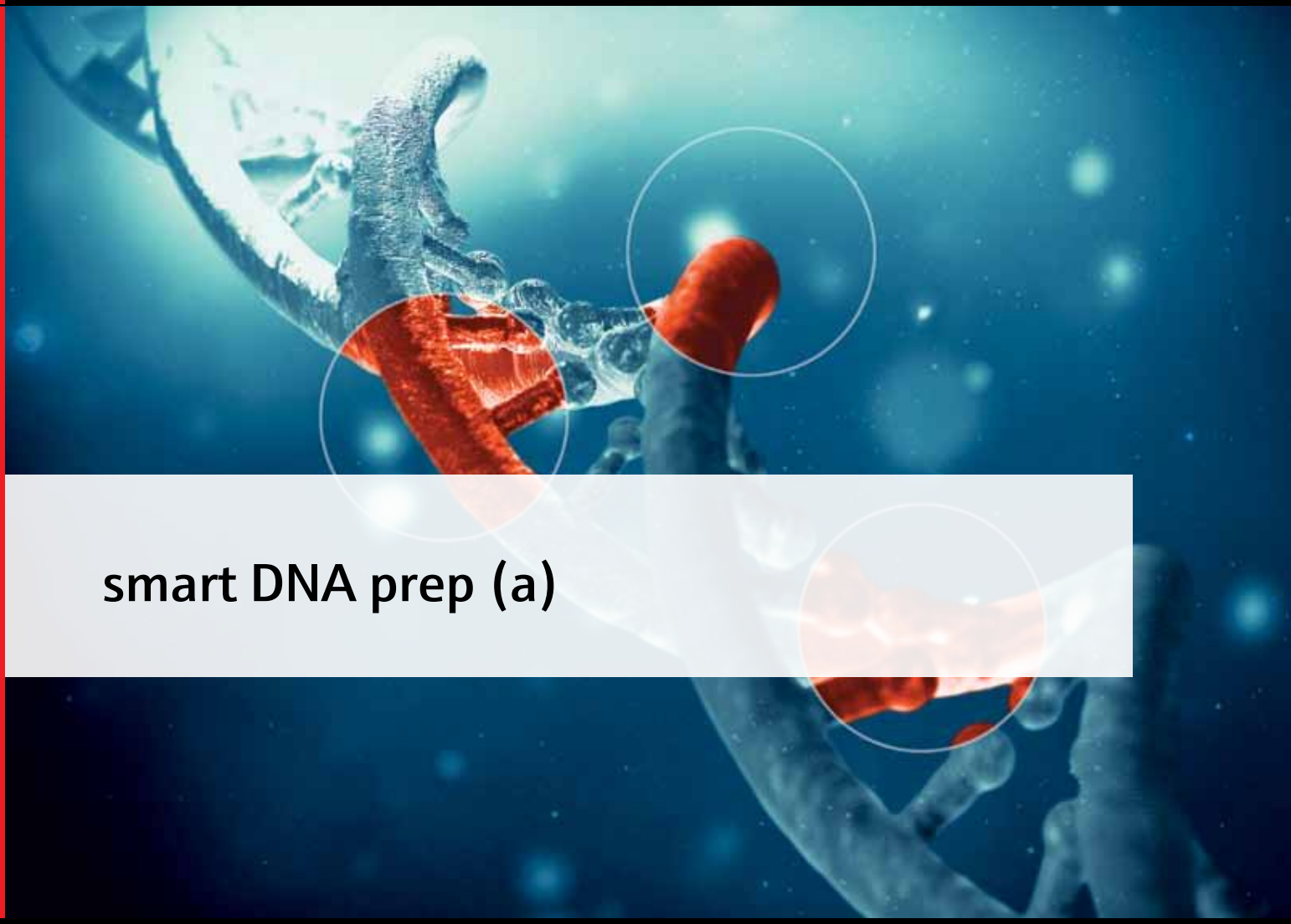
Instructions for Use
Life Science Kits & Assays



smart DNA prep (a)

analytikjena
An Endress+Hauser Company

Order No.:
845-ASS-2008016 16 reactions
845-ASS-2008096 96 reactions
845-ASP-2008016 16 reactions
845-ASP-2008096 96 reactions

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Contents

1 Introduction

1.1 Intended use

The smart DNA prep (a) 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 Analytik Jena (patent pending).

The procedure starts with the lysis of the starting material. Following lysis of the samples, lysates are transferred into the Reagent Strips or Reagent Plate of the kit, which are already prefilled with all extraction reagents needed for the automated isolation process using a unique 1 ml filter tip in combination with InnuPure® C16 / C16 *touch*, GeneTheatre or CyBio® FeliX.

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 use in subsequent downstream applications.

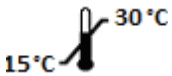
The whole extraction process just needs simple pipetting up and down. The combination of patented, low-salt DC-Technology® with patent-pending Smart Modified Surface is optimized to get a maximum of yield and quality.

CONSULT INSTRUCTION FOR USE

This package insert must be read carefully prior to 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

For easy reference and orientation, the manual 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 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" p. 5).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully prior 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 is shown.

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

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

ATTENTION!
Don't eat or drink components of the kit!
The kit shall only be handled by educated 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, please ask for the material safety data sheets (MSDS's).

3 Storage conditions

Store lyophilized Proteinase K at 4 °C to 8 °C. Divide dissolved **Proteinase K** into aliquots and storage at -22 °C to -18 °C is recommended. Repeated freezing and thawing will reduce the activity dramatically!

All other components of the smart DNA prep (a) 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 dissolve these precipitates by careful warming.

For further information see chapter "Kit components" (→ p. 9).

4 Function testing and technical assistance

The Analytik Jena AG 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 (a) kit or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. 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 (→ "Intended use") (→ "Product specifications"). Since the performance characteristics of Analytik Jena AG kits have just been validated for the application described above, the user is responsible for the validation of the performance of Analytik Jena AG kits using other protocols than those described below. Analytik Jena AG 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.

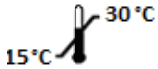
All products sold by the Analytik Jena AG are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
For research use only!

6 Kit components

	Σ 16	Σ 96
REF	845-AS[S/P]-2008016	845-AS[S/P]-2008096
SmartExtraction Tips	16	96
Proteinase K	for 1 x 1.5 ml working solution	for 3 x 1.5 ml working solution
Lysis Solution CBV	10 ml	2 x 25ml
Binding Optimizer	1 ml	5 ml
Reagent Strips L* **(* Depending on order)**	16 (pre-filled, sealed)	96 (pre-filled, sealed)
Reagent Plates L* **(* Depending on order)**	2 (pre-filled, sealed)	12 (pre-filled, sealed)
Filter Tips	1 x 16	1 x 96
Elution Tubes (0.65 ml)	16	2 x 48
Elution Caps (Stripes)	2	12
Manual	1	1
Initial steps	**Proteinase K** Dissolve by addition of **1.5 ml** of **ddH_2O**, mix thoroughly and store as described above.	**Proteinase K** Dissolve by addition of **1.5 ml** of **ddH_2O**, mix thoroughly and store as described above.

IMPORTANT
Store lyophilized **Proteinase K** at 4 °C to 8 °C.



STORAGE CONDITIONS
All other components are stored at room temperature.

6.1 Components not included in the kit

- 1.5 ml and 2.0 ml tubes
- ddH_2O
- optional RNase A (10 mg/ml)
- 1 x PBS Buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)

6.2 Components needed for isolation of nucleic acids 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(Analytik Jena AG; 845-KA-1000050)

6.3 Components needed for isolation of nucleic acids from yeasts

- Yeast Digest Buffer

  50 mM potassium phosphate

  10 mM DTT

  pH 7.5
- Lyticase (stock solution: 10 U/µl)

7 GHS Classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases
Binding Optimizer	Acetic acid 10–≤25 %	 Warning	315, 319	101, 102, 103, 280, 305+351+338, 362, 302+352, 403+233, 501
Reagent Plates/Strips L	Propan-2-ol 50–100 % Ethanol 50–100 % Hydrochloric acid ≤2.5 %	 Danger	225; 290, 319; 336	101;102;103;210;241;303+361+353;305+351+338;405;501
Proteinase K	Proteinase, Tritirachium album serine	 Danger	315, 319, 334, 317, 335	101, 102, 103, 261, 280, 305+351+338, 342+311, 405, 501

7.1 Hazard phrases

225 Highly flammable liquid and vapor.

290 May be corrosive to metals.

315 Causes skin irritation.

317 May cause an allergic skin reaction.

319 Causes serious eye irritation.

334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

335 May cause respiratory irritation.

336 May cause drowsiness or dizziness.

7.2 Precaution phrases

101	If medical advice is needed, have product container or label at hand.
102	Keep out of reach of children.
103	Read label before use.
210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
241	Use explosion-proof electrical/ventilating/lighting/equipment.
261	Avoid breathing dust/fume/gas/mist/vapors/spray.
280	Wear protective gloves/protective clothing/ eye protection/face protection.
362	Take off contaminated clothing.
405	Store locked up.
501	Dispose of contents/container in accordance with local/regional/national/international regulations.
302+352	IF ON SKIN: Wash with plenty of water.
342+311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
403+233	Store in a well-ventilated place. Keep container tightly closed.
303+361+353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
305+351+ 338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

8 Sample preparation for eukaryotic cells

8.1 Product specifications

Starting material:

- Eukaryotic cells (1×10^5–1×10^7)

8.2 Lysis of starting material

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
1×10^5–1×10^6	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 as described in “Sample preparation for eukaryotic cells” on p. 15!

NOTE
We recommend to use 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 shortly and incubate for 10 minutes at room temperature. Be sure, that the RNase A is free of DNase-activity.

Proceed with “Preparation of Reagent Plates or Reagent Strips” on p. 25.

9 Sample preparation of tissue samples

9.1 Product specifications

Starting material:

- Tissue samples (1 mg–100 mg)
- Rodent tail (0.1 cm–1 cm)

9.2 Proteolytic lysis of starting material

1. Cut the starting material into small pieces and place it 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 in 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 as described in “Sample preparation of tissue samples” on p. 17!

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. 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 the contamination of the sample with this solid phase (e.g. fat).

Proceed with “Preparation of Reagent Plates or Reagent Strips” on p. 25.

10 Sample preparation of bacteria cell pellets

10.1 Product specifications

Starting material:

- Bacteria cell pellets (1 x 10^5–1 x 10^9 cells)

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 of Lysozyme: 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. 22.

10.3.2 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)

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

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

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

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** (Analytik Jena AG; 845-KA-1000050)

The innuPREP Bacteria Lysis Booster Kit has been developed for a highly 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*.

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. 22.

10.3.3 Staphylococcus

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

Stock solution of Lysostaphin: 0.4 U/µl

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

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

Alternatively:

Use the **innuPREP Bacteria Lysis Booster** (Analytik Jena AG; 845-KA-1000050)

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*.

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. 22.

10.4 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 in 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 shortly and incubate for 10 minutes at room temperature. Be sure, that the RNase A is free of DNase-activity.

Proceed with “Preparation of Reagent Plates or Reagent Strips” on p. 25.

11 Sample preparation of yeast cell pellets

11.1 Product specifications

Starting material:

- Yeast cell pellets (1 x 10^5–1 x 10^9 cells)

11.2 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. 11).
   After resuspension start enzymatic pre-lysis as described below.

11.3 Pre-lysis of resuspended starting material

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

Stock solution of Lyticase: 10 U/µl

Add **10 µl 10 U/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. 24.

11.4 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 in a shaking platform.

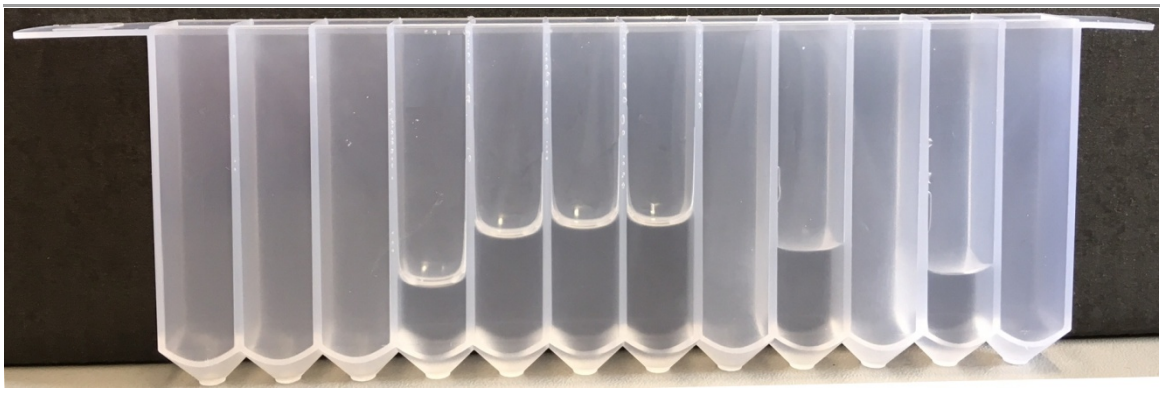
   Lysis time of 30 minutes is often sufficient to get enough DNA. If the sample is not clear after 30 minutes prolong the incubation time until the sample is clear.

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

Proceed with “Preparation of Reagent Plates or Reagent Strips” on p. 25.

12 Preparation of Reagent Plates or Reagent Strips

12.1 General filling scheme



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

12.2 Unpacking of Reagent Plates or Strips and piercing of sealing foil

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

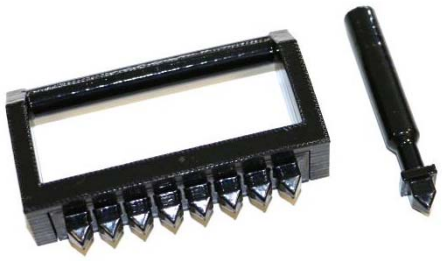
A Unpacking of Reagent Reservoirs

Reagent Reservoirs are optional delivered wrapped into plastic bags for transport protection.

Carefully open the overpack of Reagent Reservoirs by using scissors.

B Piercing of sealing foil

NOTE
Invert the Reagent Plates / Reagent Strips 3–4 times and thump it onto a table to collect the pre-filled solutions at the bottom of the wells.
Before using Reagent Plates or Reagent Strips, the sealing foil has to be pierced manually. Always wear gloves while piercing of the foil!

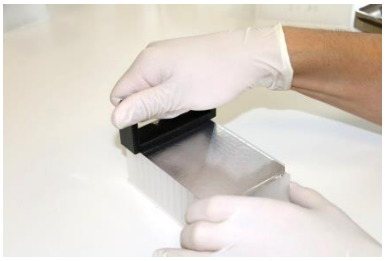
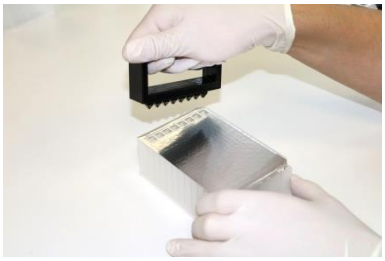
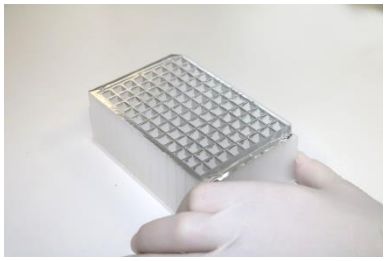


Reagent Plates / Reagent Strips are pre-filled with extraction reagents and are sealed with a foil. Prior to use, this foil has to be pierced manually, by using the piercing tools (single piercer or 8fold piercer).

Keep the Reagent Plates / Reagent Strips in a horizontal position to avoid spilling of the reagents while piercing of the foil.

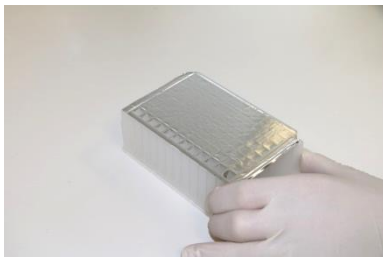
Open all cavities (one row per sample).

Using 8 samples in parallel

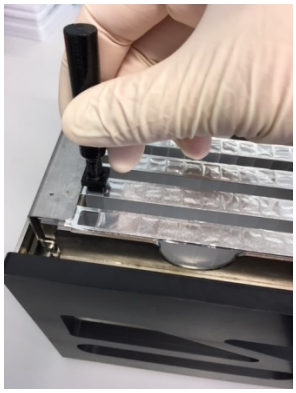
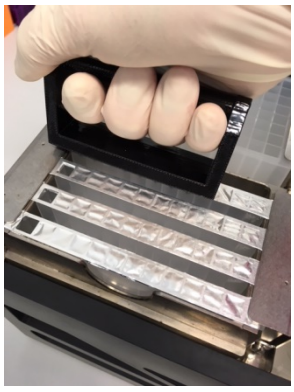




Using single samples





Using stripes


IMPORTANT

Open all cavities of one row per sample!

NOTE

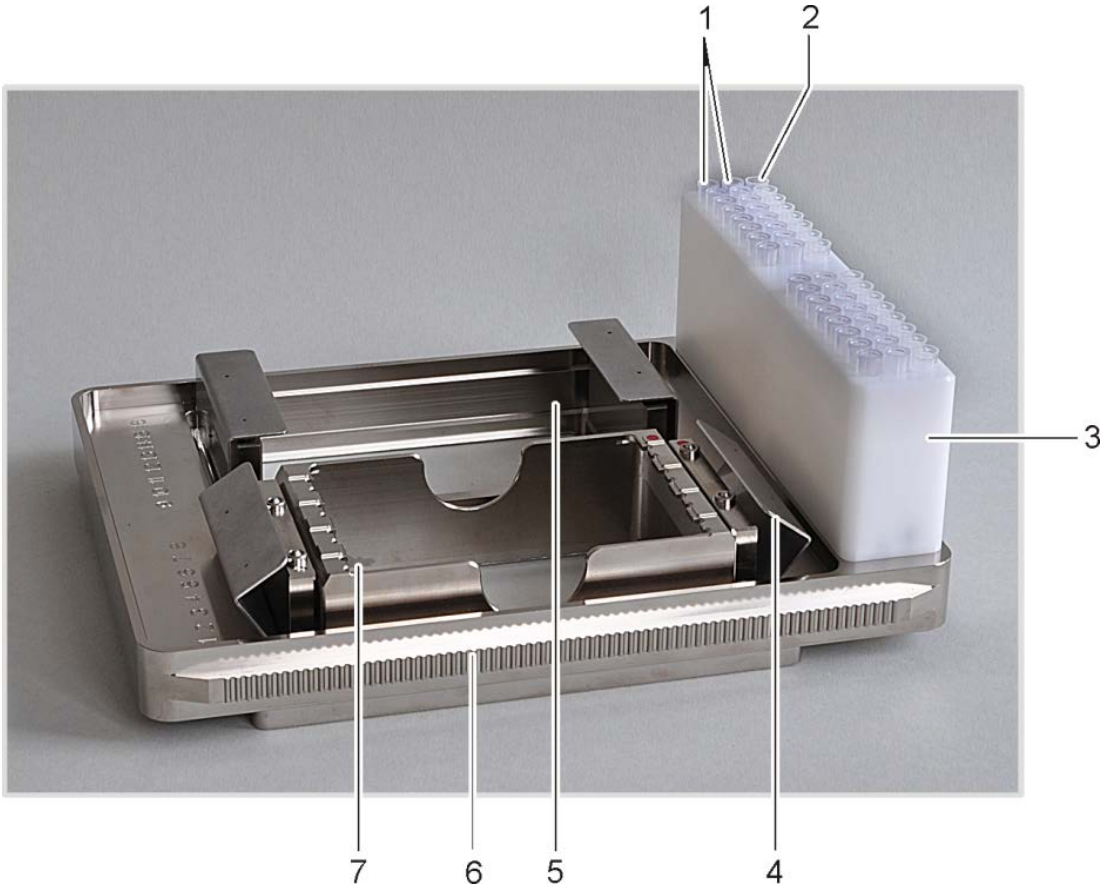
The lysed sample will be processed using a liquid handling platform. Please follow the instruction of the manual according to the following chapters:

Automated extraction using InnuPure® C16 / C16 *touch*	on p. 30
Automated extraction using GeneTheatre	on p. 41
Automated extraction using CyBio® FeliX	on p. 46

Pay special attention to sub-chapter “Loading the sample…”.

13 Automated extraction using InnuPure® C16 / C16 *touch*

13.1 Sample tray of InnuPure® C16 / C16 *touch*



No. 1:	SmartExtraction and standard filter tips
No. 2:	Elution vessels for purified samples
No. 3:	Tip block
No. 4:	Pressure pad
No. 5:	Sample block for reagent plates or adapter for reagent strips
No. 6:	Serrated guide rail (C16 *touch*: non-serrated)
No. 7:	Adapter for reagent strips

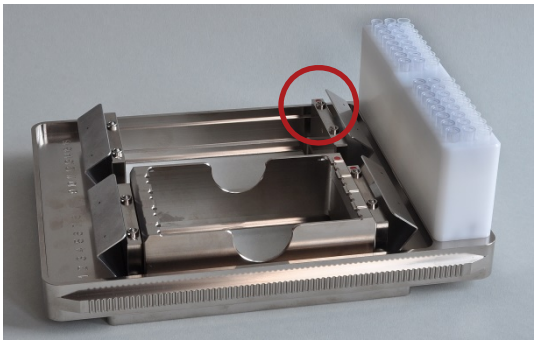
13.2 Preparing sample tray of InnuPure® C16 / C16 *touch*

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 Reagent Strips as number of samples!

1. Move the InnuPure® C16 / 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 for the 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.

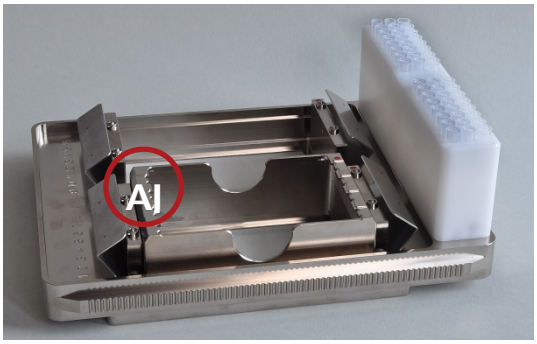
Reagent Plate

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 Reaction 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 and Reagent Strips 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. 33)

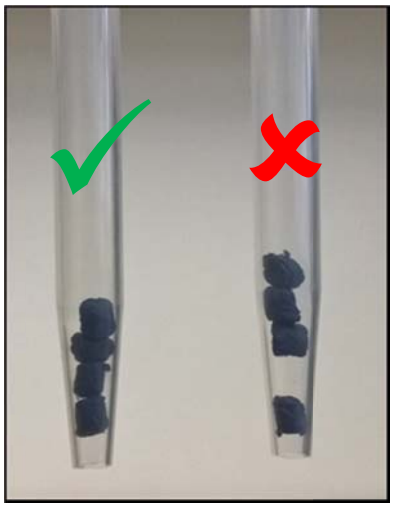
NOTE
Extracted high molecular weight DNA from large sample amounts tends to be very viscous. In order to improve the handling of DNA for downstream applications, which don't require high molecular weight DNA, extraction protocols include a homogenization step reducing the fragment size of extracted DNA. If 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 **cavities 12** of the reagent plastics at the end of the protocol. 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 a 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
Especially with the Reagent Strips, make sure that for every Reaction Strip the tips and the elution vessel are in the corresponding positions in the tip block!

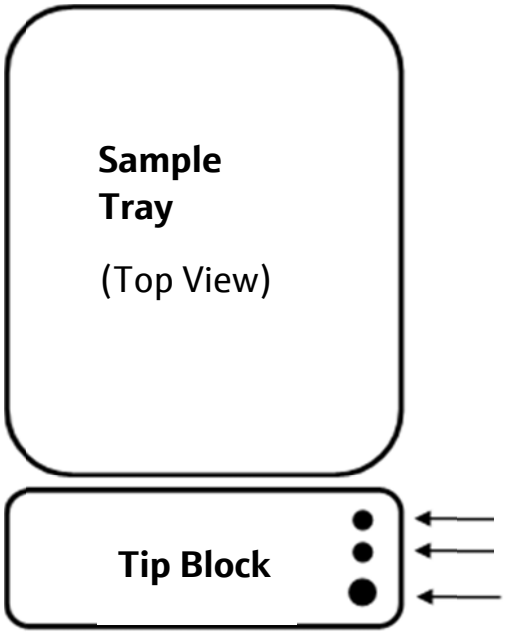
IMPORTANT NOTE
Use Elution Tubes (0.65 ml) with corresponding Elution Caps.

13.3 Handling of SmartExtraction Tips



Checking the SmartExtraction Tips.

Make sure that the Smart Modified Material is collected near the outlet of the SmartExtraction Tip. If necessary, flip the tip by finger or edge of 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 side.



Loading Pipette Tips to InnuPure® C16/C16 *touch*.

The SmartExtraction Tips are inserted in the tip row 1. The tip row 1 is the tip row adjacent to the Reagent Plates or Reaction Strips. See figure left.

13.4 Loading the sample to InnuPure® C16 / C16 *touch*

NOTE
The following step will be done after sample lysis!

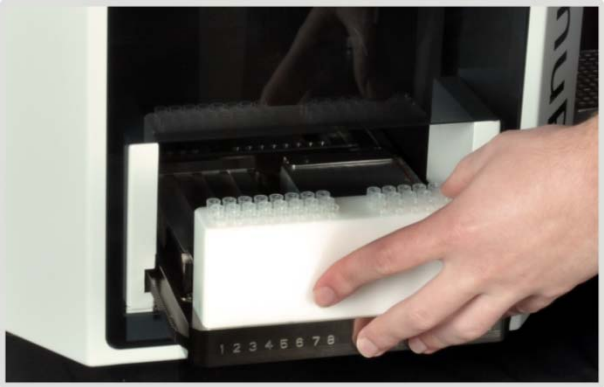
1. Prepare the Reagent Plate or Reagent Strips and sample tray according to chapter 12.

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 Reagent Strips as number of samples!

2. Transfer the whole sample into the **first cavity** (cavities which are more distant from the tip block) of Reagent Strips or Reagent Plates.
3. Transfer **40 µl** of **Binding Optimizer** to the lysed sample into the **first cavity** of Reagent Strips or Reagent Plates.

13.5 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 Strips forward into the adapter on the front 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 crushing

Immediately let go of the sample tray once it is being pulled in. Otherwise, there is a risk of your hand being crushed.

3. Start the extraction protocol:

- Press [SELECT PROTOCOL] in the starting window.
- Select the desired extraction protocol

  “SE_Ext_Lysis_C16_01” (32 minutes) or

  “SE_Ext_Lysis_Fast_C16_01” (21 minutes) or

  “SE_Ext_Lysis_Sensitive_C16_01” (67 minutes)

  and press [START].

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 ID's, press [OK] or [CANCEL].

NOTE
It is possible to enter sample ID's and to create a run log file. Find more detailed information how to start an extraction protocol using InnuPure® C16 in the user manual "6.3.5 Using the sample setup tool" on page 37!

6. After completion of the protocol press [NEXT] and the sample tray is then automatically 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 after pressing [NEXT] and the message 'Program finished' is shown on the screen of the device!

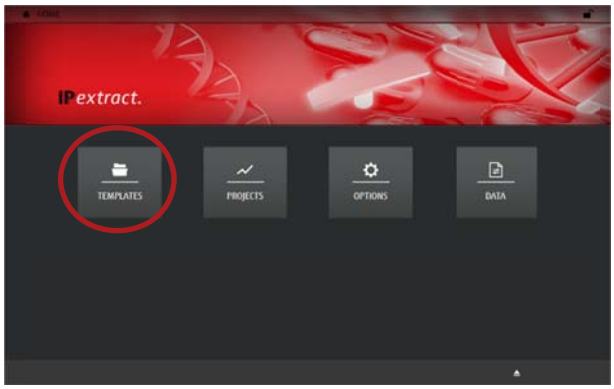
7. Remove the sample tray from the adapter of the InnuPure® C16 and move it back into the priming station.
8. After finishing the extraction protocol, the Elution Tubes (0.65 ml) contain the extracted DNA. Close the lids and store the DNA under proper conditions.

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

13.6 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*.

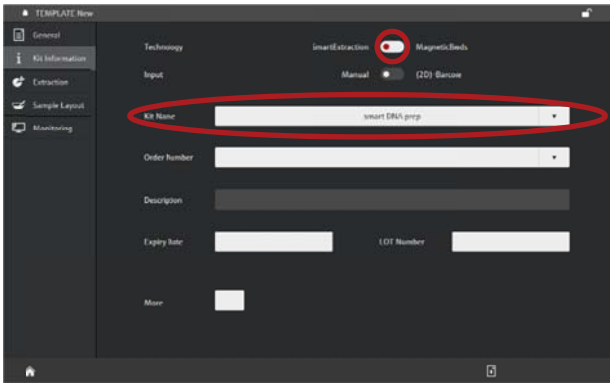
1. Switch on the InnuPure® C16 *touch* and the tablet computer. Wait until the home screen of IP*extract* is displayed on the tablet screen.



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 “Kit Name”!

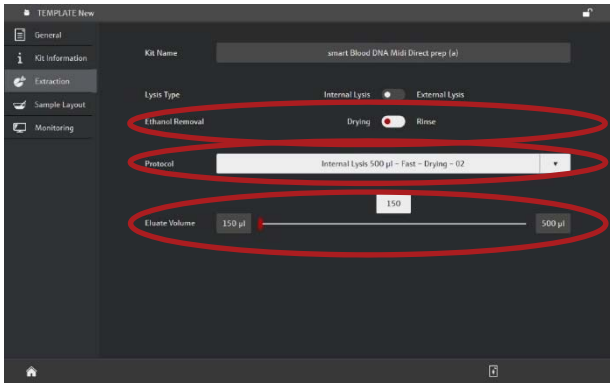


NOTE
“Kit Information” tab

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

“External Lysis – Drying – 03”	(32 minutes) or
“External Lysis – Fast – Drying – 03”	(21 minutes) or
“External Lysis – Sensitive – Drying – 03”	(67 minutes)
“External Lysis – Rinse – 03”	(28 minutes) or
“External Lysis – FAST - Rinse – 03”	(17 minutes) or
“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.

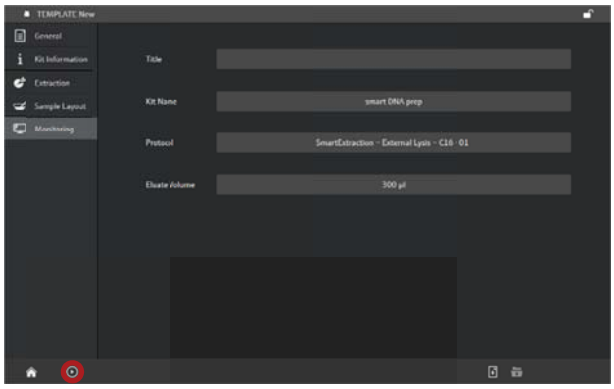
NOTE
For most applications, Ethanol Removal by “Drying” is recommended. If the extracted DNA is conceived for very ethanol-sensitive downstream applications (e.g. Droplet PCR), chose the option “Rinse”.

“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. 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
12. 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!

14 Automated extraction using GeneTheatre

14.1 Accessories needed

- 8 channel pipette head 1000 µl (844-00415-0) or optional 1 channel pipette head 1000 µl (844-00414-0)
- Tip adapter 96 / 1000 µl (OL3317-11-140)
- Optional: Adapter for Reagent Strips (845-60006-0)
- Waste Box for used tips (844-00430-0)
- Height Adapter 40 mm (844-00445-0)

14.2 Import of tube and plate data

1. Copy "Components.gttub" and "Components.gtplt" to a USB memory stick.
2. Switch on GeneTheatre and open GeneTheatre software.
3. Select menu command FILE / IMPORT / IMPORT CAVITIES and click OPEN.
4. Select cavity file "Components.gttub" from USB memory stick to import data to GeneTheatre software.
5. Select the required tubes/cavities and click PASTE.

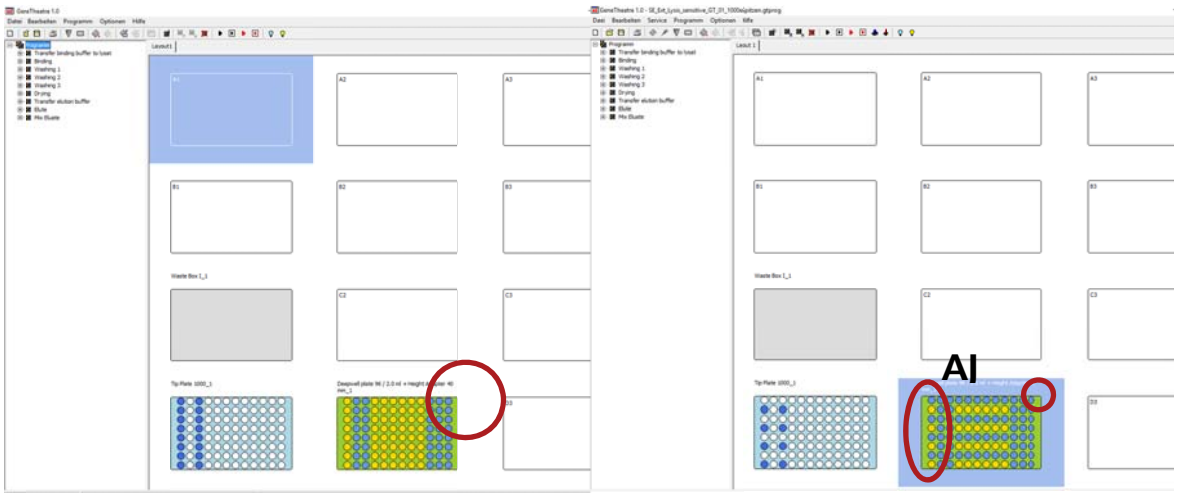
NOTE
Multiple selections are possible with the Shift or Ctrl key pressed.

6. Select menu command FILE / IMPORT / IMPORT COMPONENTS and click OPEN
7. Select cavity file "Components.gtplt" from USB memory stick to import data to GeneTheatre software.
8. Select the required plate data and click PASTE.

NOTE
Multiple selections are possible with the Shift or Ctrl key pressed.

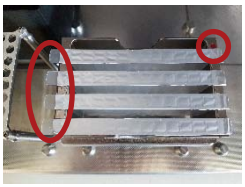
14.3 Preparing GeneTheatre

1. Place the empty waste box onto deck position C1.
2. Place the 40 mm height adapter onto deck position D2.
3. Place the Tip adapter 96 / 1000 µl onto deck position D1.
4. Place one SmartExtraction Tip per sample to the column 1 of the Tip adapter. Place one standard filter tip to column 3 of the Tip adapter.



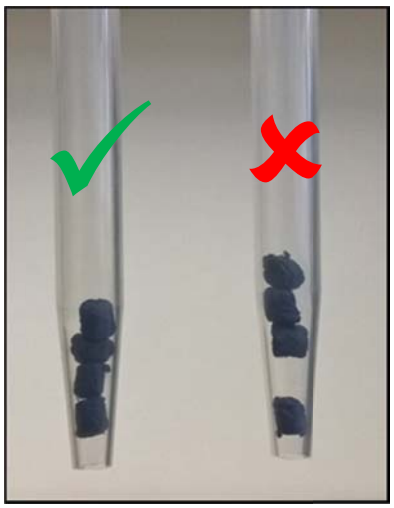
Deck layout using **Reagent Plate**

Deck layout using **Reagent Strips**



NOTE
Especially with the Reagent Strips, make sure that for every Reagent Strip the tips are in the corresponding positions in the tip adapter!

14.4 Handling of SmartExtraction Tips



Checking the SmartExtraction Tips.
Make sure that the Smart Modified Material is collected near the outlet of the SmartExtraction Tip. If necessary, flip the tip by finger or edge of 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 side.

14.5 Loading the sample to GeneTheatre

NOTE
The following step will be done after the sample lysis!

1. Prepare the Reagent Plate or Reagent Strips according to chapter 12.

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 Reagent Strips as number of samples!

2. Transfer the **sample (max. 400 µl)** into the **first cavity** (left) of Reagent Strips or Reagent Plates.
3. Transfer **40 µl** of **Binding Optimizer** to the lysed sample into the **first cavity** of Reagent Strips or Reagent Plates.
4. Place the **Reagent Plate** or optional one **adapter with Reagent Strips** onto the **40 mm height adapter (deck position D2)**.

NOTE
For Reagent Plates the notched corners of the reagent plate must be oriented to the upper corners of **deck position D2**. For Reagent Strips, put the adapter on the 40 mm height adapter at **deck position D2** in a way that the red dot on the adapter resides at the rear right corner of **deck position D2**. Put in the Reagent strips in a way that the AJ labels are oriented to the left side of the adapter (→ "Preparing GeneTheatre", p. 42).

14.6 Automatic processing of GeneTheatre

1. Switch on the GeneTheatre and open GeneTheatre software.
2. Load the extraction protocol:
   "SE_Ext_Lysis_dry_GT_01" (55 minutes) or
   "SE_Ext_Lysis_Fast_dry_GT_01" (25 minutes) or
   "SE_Ext_Lysis_Sens_dry_GT_01" (85 minutes)
3. Open pipetting step Elute and adjust Elution Volume; 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

4. After finishing the extraction protocol, the **cavity 12** of the Reagent Plastics contains the extracted DNA. Store the DNA under proper conditions.

NOTE

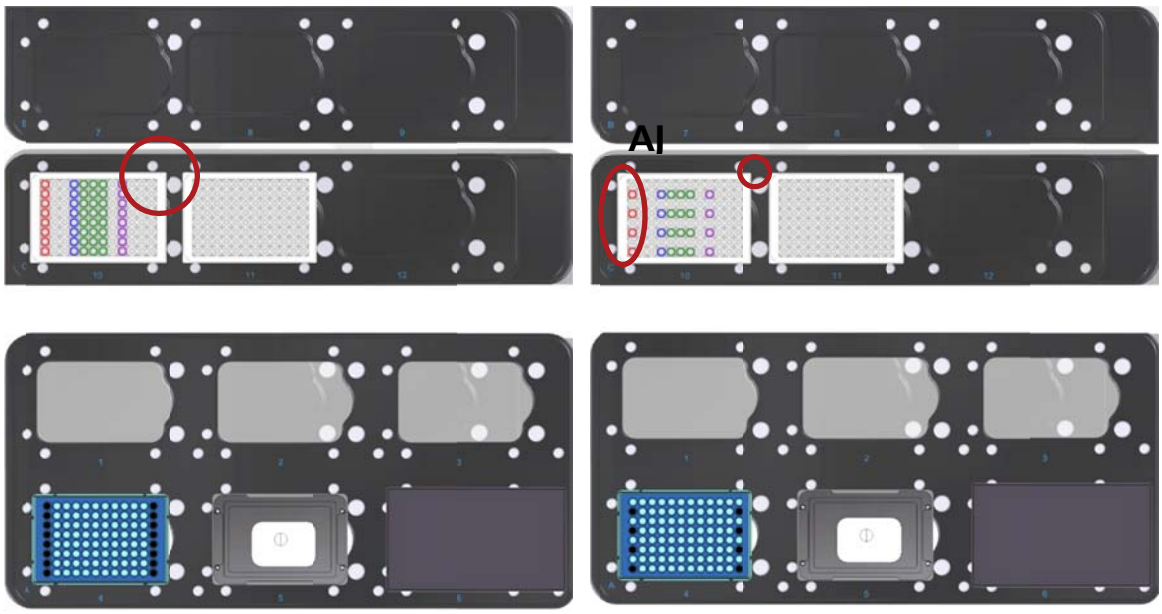
Store the DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C!

15 Automated extraction using CyBio® FeliX

15.1 Accessories needed

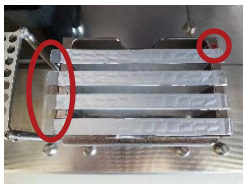
- CyBio® FeliX Basic Unit with Enclosure and CyBio® Composer Software (OL5015-24-100)
- CHOICE™ Head (OL3316-11-300) and 8-Channel CHOICE™ 10 µl – 1000 µl Adapter (OL3316-11-330)
- alternatively CyBio® FeliX Head R 96/1000 µl (OL3316-14-950) and 8-Channel Adapter; Head R 96 (OL3317-11-330)
- Tip Rack 96/1000 µl (OL3317-11-140)
- Waste Box I (small) (844-00430-0)
- Waste Bag (10-406-342)
- System Specific Pre-configured Desktop Computer (0006100-00)
- Optional: Elution Plate (1.2 ml) for InnuPure C96, 5 pieces (845-IP-0096005)
- Optional: Adapter for Reagent Strips (845-60006-0)

15.2 Preparing CyBio® FeliX



Deck layout using **Reagent Plate**

Deck layout using **Reagent Strips**



1. Place the empty **Waste Box** onto deck **position 6**.
2. Place the **8-Channel Adapter** with the **37 mm Support** onto **deck position 5** (the adapter to use depends on the pipetting head).
3. Place the **Tip Rack 96/1000 µl** onto deck **position 4**.
4. Place one **SmartExtraction Tip** per sample to **column 1** and one **Filter Tip** per sample to **column 12** of the **Tip Rack 96/1000 µl**.

NOTE

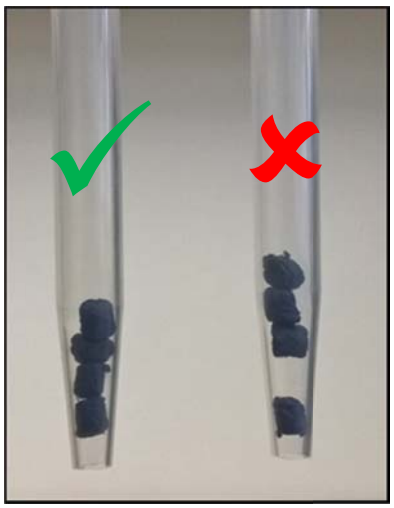
Extracted high molecular weight DNA from large sample amounts tends to be very viscous. In order to improve the handling of DNA for downstream applications, which don't require high molecular weight DNA, extraction protocols include a homogenization step reducing the fragment size of extracted DNA. If downstream application requires high molecular weight DNA, column 12 of the Tip Rack 96/1000 µl must be left empty. As a result, the eluate will remain in **column 12** of the Reagent Plastics at the end of the protocol. 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 a loss of DNA integrity pipet carefully with a wide-bore or cut tip.

NOTE

Especially with the Reagent Strips, make sure that for every Reagent Strip the tips are in the corresponding positions in the Tip Rack 96/1000 µl!

5. As a final Elution Plate (deck position 11) multiple options are possible:
   - Elution Plate (1.2 ml) for InnuPure C96, 5 pieces (845-IP-0096005)
   - Micronic 750 µl pre-capped and racked 2D-tubes (MP52706-Y20)
   - Greiner Cryo.S 600 µl pre-racked (977561, 977580)

15.3 Handling of SmartExtraction Tips



Checking the SmartExtraction Tips.
Make sure that the Smart Modified Material is collected near the outlet of the SmartExtraction Tip. If necessary, flip the tip by finger or edge of 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 side.

15.4 Loading the sample to CyBio® FeliX

NOTE
The following step will be done after the sample lysis!

1. Prepare the Reagent Plate or Reagent Strips according to chapter 8.

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

2. Transfer the whole **sample (max. 400 µl)** into the **first cavity** (left) of Reagent Strips or **first column** of Reagent Plate.
3. Transfer **40 µl** of **Binding Optimizer** to the lysed sample into the **first cavity** of Reagent Strips or **first column** of Reagent Plate.
4. Place the opened Reagent Plate or optionally one Strip Adapter with Reagent Strips onto **deck position 10**.

NOTE
For Reagent Plates the notched corners of the Reagent Plate must be oriented to the upper corners of deck position 10. For Reagent Strips, put the Strip Adapter on deck position 10 in a way that the red dot of the adapter resides at the rear right corner of deck position 10. Put in the Reagent Strips in a way that the AJ labels are oriented to the left side of the adapter (→ "Preparing CyBio® FeliX", p. 47).

15.5 Automatic processing of CyBio® FeliX

1. Switch on the CyBio® FeliX and open Composer.
2. Load the extraction protocol:

   "SE_Ext_Lysis_dry_FX_8_01" (38 minutes) or

   "SE_Ext_Lysis_Fast_dry_FX_8_01" (23 minutes)
3. Adjust parameter elution volume ($(Vol_elute)) in the headline of the protocol as recommended in table below; press [OK] and start the protocol.

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

NOTE
The chosen protocol is performed by device and after the protocol is finished the message "Purification process completed" is shown in the screen of the computer!

4. After finishing the extraction protocol, the eluate will be in the final elution plate in column 1 on **deck position 11**. If high molecular weight DNA is required and consequently standard 1 ml filter tips were not placed in Tip Rack 96/1000 µl, the eluate will remain in cavities 12 of **Reagent** Strips or column 12 of the Reaction Plate on **deck position 10**.
5. After finishing the extraction protocol, remove and discard the used Deep Well Plates and the used tips.

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

16 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	Flip the Pipette Tip by finger or edge of table or invert the Pipette Tip a few times to collect Granulates at the lower part of pipette tip.
Preparation without Binding Optimizer	It is important to add the Binding Optimizer to the Reagent Plastic as described in chapters for handling of the liquid handling platforms. Binding Optimizer need 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 insufficient	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	RNase A digestion

17 Related Products

Name	Amount	Order No.
Additional products for nucleic acid purification		
innuPREP Bacteria Lysis Booster	50 rxn	845-KA-1000050
innuPREP Proteinase K	6 mg	845-CH-0010006
	30 mg	845-CH-0010030
Automated nucleic acid purification		
smart Blood DNA Midi prep (a)	16 rxn (Strips)	845-ASS-1208016
	96 rxn (Strips)	845-ASS-1208096
	16 rxn (Plates)	845-ASP-1208016
	96 rxn (Plates)	845-ASP-1208096

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