MolYsis-SelectNA™*plus*

Automated sample pre-treatment, bacterial/ fungal DNA isolation

Body fluids

(e.g. *ascites, BAL, blood, CSF, joint aspirates, plasma, synovial fluid, urine*)

Swabs

(e.g. mouth, nasopharynx, wounds, bones)

Tissues

(e.g. *abscesses*, *biopsies, heart valves, pacemaker*)

– For research use only –
– Not for *in-vitro* diagnostic use –

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Kit Information

Kit Contents – MolYsis-SelectNA™plus

Automated DNA Isolation	**48 rxn (D-450-048)**
Kit 1 – Buffers & Consumables (store at +18 to +25°C)	
A) Sample Dilution & Tissue Pre-treatment Buffers, in rack	
SU	2x 25 ml
TSB	2x 25 ml
PKB	2x 7.5 ml
B) Cartridges & Consumables	
ST – Sample tubes, 2.0 ml, flip cap tubes for tissues & swabs pre-treatment only, in DNA-free bags	1x 50
Plus-SV – Plus-Sample vials; screw cap vials for instrument, 2.0 ml, in DNA-free bags	4x 12
Extraction columns, in DNA-free bags	4x 12
Extraction cartridges, in trays	4x 12
MolYsis-SelectNAplus Buffer cartridges, pre-filled, in trays	4x 12
ET – Elution tubes, 1.5 ml, in DNA-free bags	4x 12
Kit 2 – Enzymes (store at -15 to -25°C), in white boxes	
2A) *MolDNase C*, solution, red cap, in bags	4x [12x 0.05 ml]
2B) *Proteinase K*, solution, blue cap, in bags	4x [12x 0.04 ml]
2C) *BugLysis plus*, solution, yellow cap, in bags	4x [12x 0.02 ml]
2D) *Enzyme K,* solution, in bags	4x [3x 0.08 ml]
Manual (in Kit 1)	
Manual	1x
Short manual sheets	4x

Symbols

Symbols used in labelling and in section ‘Hazard and Precautionary Statements’ (pages 8 to 9).

	Content of the package	LOT	Batch code		Toxicity
	Manufactured by	REF	Catalogue number		Harmful to health
	Use by		Flammable		Environmentally Hazardous
	Temperature limitation (store at)		Irritant		
Cont.	Consult instructions for use		Corrosive		

Storage and Stability

Guarantee for ***full performance*** of reagents and buffers is given through the ***expiration date*** printed on the label at the outer box, if the ***packed material is undamaged*** upon arrival and the reagents are unopened. Guarantee for full performance of ***MolYsis-SelectNA™plus*** as specified in this manual is only valid if storage conditions are followed.

Kit 1 (Buffers & Consumables): Buffers, cartridges and consumables must be stored dry, dark and at room temperature (+18 to +25°C).

Kit 2 (Enzymes): Take care that the vials of the DNA Isolation unit have to be stored at -15 to -25°C upon delivery.

Once opened, the vials have to be used as specified by the protocol.

Intended Use and Indication

MolYsis-SelectNA™*plus* is a kit for the isolation of microbial DNA (bacterial/fungal). **MolYsis-SelectNA™*plus*** is intended as a kit for ***research* use only** for body fluids, swabs and for tissues (validated specimens see Tab. 4, page 13). The kit is for laboratory use (professional users).

Contraindication

MolYsis-SelectNA™*plus* kit is not intended to be used as in vitro diagnostic test for the detection and identification of any specific pathogen.
MolYsis-SelectNA™*plus* is not indicated to be used for pathogens with safety level S3 and S4. An exemplary selection is listed in Tab. 1.

Tab. 1: Contraindication of **MolYsis-SelectNA™*plus*** for pathogens with safety level S3 and S4 (exemplary selection).

Bacillus cereus biovar anthracis	*Mycobacterium microti*
Coxiella burnetii	*Mycobacterium pinnipedii*
Brucella abortus (B. melitensis biovar abortus)	*Mycobacterium tuberculosis (Mycobacterium tuberculosis subsp. tuberculosis)*
Brucella canis (B. melitensis biovar canis)	*Mycobacterium ulcerans*
Brucella inopinata	*Orientia tsutsugamushi (Rickettsia tsutsugamushi)*
Brucella melitensis (B. melitensis biovar melitensis)	*Rickettsia africae*
Brucella neotomae (B. melitensis biovar neotomae)	*Rickettsia akari*
Brucella ovis (B. melitensis biovar ovis)	*Rickettsia australis*
Brucella suis (B. melitensis biovar suis)	*Rickettsia conorii*
Burkholderia mallei (Pseudomonas mallei)	*Rickettsia heilongjiangensis*
Burkholderia pseudomallei (Pseudomonas pseudomallei)	*Rickettsia japonica*
Chlamydia psittaci (Chlamydophila psittaci)	*Rickettsia prowazekii*
Coxiella burnetii	*Rickettsia rickettsii*
Escherichia coli (enterohemorrhagic (EHEC) Strains O157:H7 or O103)	*Rickettsia sibirica*
Francisella tularensis subsp. tularensis	*Rickettsia typhi*
Mycobacterium africanum	*Salmonella Typhi*
Mycobacterium bovis	*Shigella dysenteriae*
Mycobacterium caprae (Mycobacterium tuberculosis subsp. caprae)	*Yersinia pestis*
Mycobacterium leprae	

Product Use Limitations

Usage of **MolYsis-SelectNA™*plus*** reagents for ***research* use only**! Whole blood samples must be collected and stabilized using either EDTA or citrate.
MolYsis-SelectNA™*plus* is not intended for non-primary sterile specimen materials, frozen and thawed specimen materials, nor for highly viscous specimen materials. Not for other specimens than mentioned above. Cells must be intact for reliable results. This requires specimens not to be stored in solutions, which induce cell lysis. Transport media including Agar, gel, charcoal medium and Amies hold a risk of inhibiting the amplification or clogging the extraction columns and should be avoided.

Apparatuses and Consumables to be Supplied by the User

The following equipment, consumables and reagents not supplied with this kit are recommended to be used.
Do not transfer supplies (e.g., pipettes, microcentrifuges, vortexer, racks) and disposable material as specified by the handlings below from one working place to another.

Necessary equipment for the instrument:

- **SelectNA™*plus*** DNA extraction instrument (Molzym order no. D-400-001).
- ***Pipette tips* SelectNA™*plus***, DNA-free (Molzym order no. D-925-024 / D-925-048 / D-925-096).
  Use only Molzym´s DNA-free *Pipette tips* for the SelectNA™*plus*.
- ***Waste bags* SelectNA™*plus***, (Molzym order no. D-928-500).

Sample preparation:

- 1x UV Class II biological safety cabinet
- 1x vortexer, e.g., VWR, Darmstadt, Germany
- 1x thermomixer (2.0 ml tubes), e.g., Eppendorf comfort, Eppendorf
- 1x low speed mini-centrifuge (e.g., MiniFuge, VWR, Darmstadt, Germany) or a bench top microcentrifuge (e.g., miniSpin, Eppendorf, Germany)
- Precision pipette up to 20 µl, up to 200 µl and up to 1000 µl, e.g., Eppendorf, Germany
- Sterile forceps for *Extraction columns* loading
- Sample racks

<u>Only tissue protocol</u>

- Sterile forceps
- Sterile support, e.g., Petri dish
- Sterile scalpel or sterile preparation scissors

Plastic Consumables and reagents:

- DNA-free pipette tips (with aerosol filter), e.g., Biosphere® plus, Sarstedt, Germany
  - 100 µl type Eppendorf (70.760.212)
  - 300 µl type Eppendorf (70.3040.255)
  - 1000 µl type Eppendorf (70.3050.255)
- Surface decontamination, e.g., Meliseptol® New Formula (rapid disinfectant, ethanol containing), B. Braun, Germany (19758) or an ethanol containing disinfectant for cleaning of the SelectNA™*plus* instrument
- Cleaning of the waste chute: Mildly alkaline cleaning powder for special washing machines with sodium hydroxide, e.g. LABWASH® Premium Classic, VWR Chemicals (84548.410)
- Cleaning of the pipetting tubes: 1 % (active Cl_2) sodium hypochlorite solution, prepared from e.g., sodium hypochlorite 14 % Cl_2 in aqueous solution, VWR Chemicals (27900.296)
- Autoclaved deionized water (121°C, 1bar, 30 min) for the pipetting tubes.
- Disposables
  - Lab coat, e.g., VWR, Germany
  - Sterile gloves, e.g., Kimberly-Clark, Germany
  - Sterile sleeves, e.g., Cardinal Health, Ireland
  - Bouffant covers, e.g., VWR, Germany
  - Hygiene mask, e.g., VWR, Germany
  - Overshoes, e.g., hygi, Germany
- Waste container for plastics and liquid waste, autoclavable

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable sleeve covers, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS) which are available on request.

CAUTION: Never add hypochlorite (bleach) or acidic solutions directly to the cartridges waste.

The lysis buffer (W0) and binding buffer (W6) are pre-filled in the *Buffer cartridges*. These buffers contain guanidine hydrochloride and guanidinium thiocyanate, respectively, which can from highly reactive compounds and toxic gases when combined with hypochlorite or other acidic solutions. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 70 % (v/v) ethanol. This kit is to be used only by skilled personnel trained for handling infectious material and molecular biological methods. Aerosols created during the extraction process in the SelectNA™*plus* instrument could contain pathogens. Therefore, the opening of the door can be a risk for the user. In the end of the extraction process a 5 min UV step is implemented for more safety of the user. The UV step reduces this risk. Nevertheless, suitable protective clothing is essential when working in the instrument.

To avoid false analytic results by DNA contamination of reagents and infection of the user by infectious agents during handling, always wear sterile protective gloves, sterile disposable sleeve covers, a lab coat, protective goggles and disposable overshoes. Work in a Class II biological safety cabinet irradiated with UV before starting according to the instruction manual of the manufacturer. The UV lamp must be switched off during working. Follow the instructions of the manufacturer for maintenance of the workstation. Irradiate the SelectNA™*plus* using the instrument's programme for UV decontamination after each extraction run (see section 1D, page 35). Dispose of potentially infectious material and the waste including cartridges and vials following national directives of the health organisation (e.g., in Germany: Vollzugshilfe zur Entsorgung von Abfällen aus Einrichtungen des Gesundheitsdienstes, 2021).

Separate *Material Safety Data Sheets* are available on request. Molzym reserves the right to alter or modify the product to improve the performance of the kit.

Hazard and Precautionary Statements

Buffer *PKB*

Contains sodium dodecyl sulfate (< 10 %):
Acute toxicity (oral, inhalation), irritation (skin and eye).

Warning

Hazard and precautionary statements*(page 9):
H302-H315-H319-H332; P280-P301+P312-P304+P340+P312-P305+P351+P338

Proteinase K, Enzyme K

Contains *Proteinase K* (≥1 %):
Respiratory sensitization and skin sensitization.

Danger

Hazard and precautionary statements*(page 9):
H317-H334;
P280-P302+P352-P333+P313-P363

BugLysis plus

Contains 2-mercaptoethanol (<10 %): **Acute toxicity (skin), eye damage, skin sensitization, reproductive toxicity and hazardous to aquatic environment (chronic).**

Danger

Hazard and precautionary statements*(page 9):
H310-H317-H318-H361d-H411;
P273-P280-P301+P310-P302+P352+P310-P305+P351+P338

Lysis buffer, prefilled in *Buffer cartridges* (W0)

Contains guanidine hydrochloride (>10 %):
Acute toxicity (oral) and irritating (eyes and skin).

Warning

Hazard and precautionary statements*(page 9):
H302-H315-H319;
P301+P312-P302+P352-P305+P351+P338

Enzyme buffer, prefilled in *Buffer cartridges* (W5)

Contains sodium dodecyl sulfate (< 10 %):
Acute toxicity (oral, inhalation), irritation (skin and eye).

Warning

Hazard and precautionary statements*(page 9):
H302-H315-H319-H332;
P280-P301+312-P304+340+312-P305+351+338

Binding buffer, prefilled in *Buffer cartridges* (W6)

Contains 2-propanol (<40 %); guanidinium thiocyanate (>10 %): **Flammable liquids, acute toxicity (oral, skin), skin corrosive and irritating (eyes), specific target organ toxicity (single exposure) and hazardous to aquatic environment (chronic)**.

Danger

Hazard and precautionary statements*:
H225-H302-H312-H314-H319-H336-H412-EUH032;
P210-P233-P280-P303+P361+P353-P305+P351+P338-P310-P362+P364

Washing buffer, prefilled in Buffer cartridges (W7)

Contains ethanol (>50 %): **Flammable liquids and irritating (eyes)**.

Danger

Hazard and precautionary statements*:
H225-H319;
P210-P233-P305+P351+P338

Emergency information (24-hours service)

For emergency medical information, please contact the regional poison center in your country.

* **H225:** Highly flammable liquid and vapour; **H302:** Harmful if swallowed; **H310:** Fatal in contact with skin; **H312:** Harmful in contact with skin; **H314:** Causes severe skin burns and eye damage; **H315:** Causes skin irritation; **H317:** May cause an allergic skin reaction; **H318:** Causes serious eye damage; **H319:** Causes serious eye irritation; **H332:** Harmful if inhaled; **H334:** May cause allergy or asthma symptoms or breathing difficulties if inhaled; **H336:** May cause drowsiness or dizziness; **H361d:** Suspected of damaging the unborn child; **H411:** Toxic to aquatic life with long lasting effects; **H412:** Harmful to aquatic life with long lasting effects; **EUH032:** Contact with acids liberates very toxic gas.

P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking; **P233:** Keep container tightly closed; **P273:** Avoid release to the environment; **P280:** Wear protective gloves/protective clothing/eye protection/face protection; **P310:** Immediately call a POISON CENTER/doctor; **P301+P310:** IF SWALLOWED: Immediately call a POISON CENTER/doctor; **P363:** Wash contaminated clothing before reuse; **P301+P312:** IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell; **P302+P352:** IF ON SKIN: Wash with plenty of water; **P302+P352+P310:** IF ON SKIN: Wash with plenty of water. Immediately call a POISON CENTER/doctor; **P303+P361+P353:** IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower]; **P304+P340+P312:** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell; **P305+P351+P338:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing; **P333+P313:** If skin irritation or rash occurs: Get medical advice/attention; **P362+P364:** Take off contaminated clothing and wash it before reuse.

Introduction

System Description

MolYsis-SelectNA™*plus* is a molecular tool for the fully automated procedure of pathogen enrichment and isolation of microbial DNA from body fluids, swabs and tissue specimens. **MolYsis-SelectNA™*plus*** is used in combination with the **SelectNA™*plus*** instrument which provide a new, completely automated solution for the depletion of host DNA, enrichment of microorganisms from clinical samples and the isolation of pure microbial DNA.

Pathogen-directed PCR can be severely disturbed or even inhibited by a high background of host DNA. Molzym has developed a technology, *MolYsis™,* which comprises a procedure for the degradation of host DNA before extraction and purification of microbial target DNA from human or animal samples.

The *MolYsis*™ technology, which is the basis of the **MolYsis-SelectNA™*plus*** kit significantly increases the detection sensitivity by amplification methods (Hansen W.L.J. et al. 2009). The enhancement of analytical sensitivity brought about by *MolYsis*™ technology translates to acceptable diagnostic values as regards bacteraemia and fungaemia (Wellinghausen N. et al. 2009). Molzym's fully automated DNA extraction solution, **MolYsis-SelectNA™*plus***, guarantees high detection sensitivity using PCR, Real-Time PCR, Next Generation Sequencing and other molecular methods for the direct analysis of bacteria and fungi in whole blood, other body fluids, swabs and tissue material.

Molzym supplies DNA-free Taq DNA polymerase (*MolTaq 16/18S* order no.: P-019-0100) and ready-to-use mastermixes as well as sequencing primers (order no.: S-775-100, bacteria or S-785-100, fungi) to run PCR assays and sequencing reactions directed to the detection and identification of pathogens (www.molzym.com). An online tool for strain identification using a BLAST algorithm with more than 7,000 quality-checked sequences of bacteria, yeasts and *Aspergillus* species is available (www.sepsitest-blast.com).

Test Principle

The **MolYsis-SelectNA™*plus*** kit supplies all reagents and consumables (excluding pipette tips, waste bags) for the automated extraction of microbial DNA from clinical samples. The **MolYsis-SelectNA™*plus*** kit is used with the **SelectNA™*plus*** instrument for the isolation of bacterial and fungal DNA from 1_ml EDTA or citrate-stabilised blood, other body fluid samples, platelet concentrates and swabs. For tissue biopsies a short manual protocol precedes the automated extraction of microbial DNA. After deposition of the sample into the instrument, the following protocol proceeds fully automated.

The pre-analytic procedure is based on the following four steps A to D (Fig. 1):

A In a first step, the sample (1 ml) is treated with a chaotropic buffer which lyses the host but not microbial cells and degrades the released host DNA (and any potentially present floating DNA from dead microorganisms) by a DNase. The lysate is liquefied and then passed through a filter column by vacuum filtration which retains microorganisms potentially present in the sample on the filter.

B In a series of following steps the immobilized microbes are washed and lysed by enzymatic treatment.

C In the following steps the microbial DNA is bound on the filter and washed.

D Finally, the microbial DNA is eluted with 200 µl elution buffer. At the end, a microbial DNA preparation is available which is depleted of host DNA and can be used for molecular analysis.

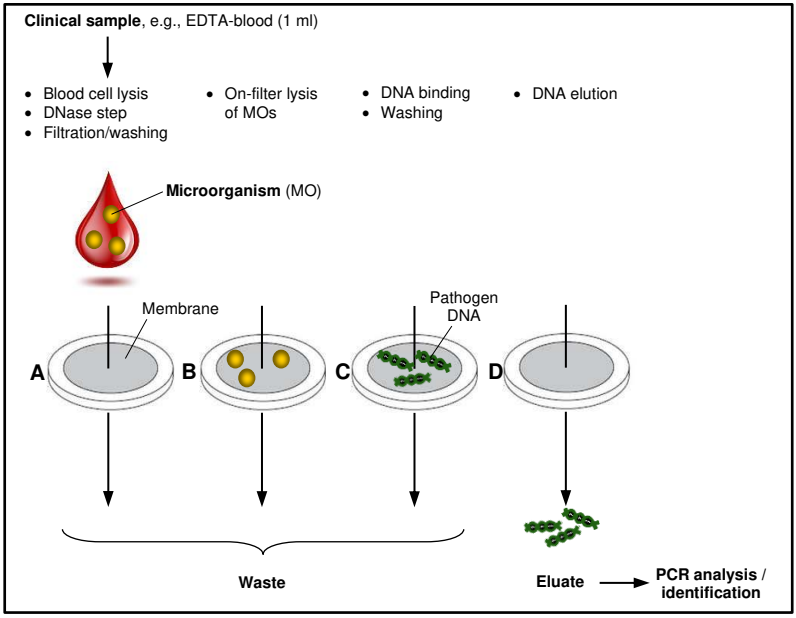

Fig. 1: Scheme of the fully automated **MolYsis-SelectNA™*plus*** procedure.

Validation

Analytical Sensitivity

Molzym's sample pre-treatment and DNA isolation constitutes the optimal solution for high sensitivity PCR and Real-Time PCR analysis of DNA from bacteria and fungi.
The analytic system used was Molzym´s *Mastermix 16S Complete* (bacteria; order no.: S-020-0100) and *Mastermix 18S Complete* (fungi; order no.: S-070-0100). By this combination, for instance, *Staphylococcus aureus* can be detected reliably at 50cfu/ml by the universal PCR assay. Spiking experiments using negative samples and serial dilutions of cultured strains of clinical isolates showed the detection limits indicated in Tab. 2.

Tab. 2: Analytical sensitivity of **MolYsis-SelectNA™*plus***.
Titre resulting in positive results from 4 to 12 repeated extractions of buffer samples spiked with strains. Analysis: Real-Time PCR (5 µl eluate/assay; Assays: *Mastermix 16S Complete*, bacteria and *Mastermix 18S Complete*, fungi; Molzym) with melting curve analysis. Bold: Limit of detection at 100 % positives lowest dilution.

Strain	cfu/ml detected (positive result)													
Gram-negative bacteria														
Escherichia coli			**1,000**	100%	500	92%	250	78%	100	50%				
Klebsiella pneumoniae	5,000	100%	1,000	100%	500	100%	**250**	100%	100	75%				
Pseudomonas aeruginosa					**500**	100%	250	75%						
Gram-positive bacteria														
Enterococcus faecalis			200	100%	**100**	100%	50	25%	25	75%	5	0%		
Staphylococcus aureus	500	100%	**250**	100%	100	83%	50	67%						
Streptococcus agalactiae					100	100%			10	100%	5	100%	**1**	100%
Fungi														
Candida albicans									5	100%	**2,5**	100%	1	67%
Candida glabrata					20	100%	10	100%	**5**	100%			1	75%
Candida krusei	**100**	100%	50	75%	20	75%								
Candida parapsilosis					20	100%	10	100%	**5**	100%	2,5	67%		
Candida tropicalis					20	100%	10	100%	5	100%			**1**	100%

Clinical Evaluation

A variety of Gram-negative, Gram-positive and fungal organisms were identified in a survey of 80 clinical samples using **MolYsis-SelectNA™*plus*** in combination with Molzym´s *Mastermix 16S Complete* (bacteria) and *Mastermix 18S Complete* (fungi). Detected organisms in clinical samples see Tab. 3 and Tab. 4.

Tab. 3: Microorganisms identified in clinical evaluations or tested in spikings*.

Gram-negative bacteria	Gram-positive bacteria	Fungi
Acinetobacter baumannii	*Bacillus subtilis**	*Candida albicans*
Escherichia coli	*Clostridium bifermentans*	*Candida glabrata*
Fusobacterium nucleatum	*Enterococcus faecalis**	*Candida krusei**
Haemophilus influenzae	*Enterococcus faecium*	*Candida parapsilosis*
*Klebsiella sp.**	*Lactobacillus sp.*	*Candida tropicalis**
*Moraxella catarrhalis**	*Staphylococcus aureus*	
Neisseria meningitidis	*Staphylococcus epidermidis*	
Pantoea agglomerans	*Staphylococcus lugdunensis*	
Providencia stuartii	*Streptococcus agalactiae**	
Raoultella planticola	*Streptococcus gallolyticus*	
*Shigella flexneri**	*Streptococcus pneumoniae*	
	Streptococcus oralis	

Tab. 4: **MolYsis-SelectNA™*plus*** is validated with the following specimens.

Fluid samples	Pericardial effusion	Biopsy sphenoid sinus
Ascites aspirates	Urine	Bone marrow
BAL	**Swabs**	Heart valve biopsies
Blood	Bones	Hematoma
Blood cultures	Mouth	Lung tissues
CSF aspirates	Nasopharynx	Mucus
Joint aspirates	Wounds	Pacemakers
Liver abscess puncture fluid	**Tissue samples**	Paraffin blocks
Plasma	Abscesses	Pericard
Platelet concentrates	Aorta	Prosthesis
Pleural fluid	Artificial tissues	(e.g. heart valve, …)

Do not use other specimens than validated in Tab. 4.

In particular, the following sample materials are inappropriate for the **MolYsis-SelectNA™*plus*** protocol:

- Gelatinous samples (e.g., sputum)
- Cell cultures
- Blood cultures with activated carbon
- Swabs on agar gel media
- Samples with transport media including Agar, gel, charcoal medium and Amies

These materials may clog the Pipette tips and Extraction columns which will cause the Pressure Monitoring System (pages 21 to 22) to reject the sample to prevent an overflow and contamination of the instrument and other samples.

Avoidance of DNA Contamination

Care should be taken to avoid DNA contamination from exogenous sources. This includes the complete pathway from sample collection to analysis. Also, it is important to minimise cross-contamination from sample to sample. For guidance see Roth et al. (2001) and Espy et al. (2006). A short summary of precautions is given below:

- **Guidelines**:
  The guidelines of the national health organisations, e.g., Robert-Koch-Institute (Germany), for sample collection, including sterilisation of the skin should be followed.

- **Decontamination**:
  Generally, use places decontaminated from DNA for handling. We recommend performing handling steps under UV-irradiated workstations. UV irradiation must be done before working according to the recommendations of the manufacturer. Routinely treat the surfaces of the working places with a commercial DNA decontamination reagent which is compatible with sterile protective gloves. Make sure that the material to be decontaminated is resistant to such treatment. Do not transport supplies (e.g., pipettes, microcentrifuges, vortexer) and disposable material as specified by the handlings below from one working place to another. The place near the instrument should be equipped with a freezer (-15 to -25°C) for storage of the enzymes of the kit. The Class II biological safety cabinet should stand in the same room, optimally beside or near the instrument.
  Always UV-decontaminate the instrument after usage. Follow the instructions given below (section 1D, page 35).

- **Infectious material and cross-contamination**:
  Handle potentially infectious material with great care and work under a Class II biological safety cabinet in order to protect yourself from infection and to avoid cross-contamination of samples and carry over contamination of buffers *SU*, *PKB* and *TSB*. Wear a disposable lab coat, sterile protective gloves, sterile disposable sleeve covers, protective goggles and a disposable mask at any handling step, particularly when handling infectious material. Take care to open the enzyme vials (Kit 2, vials 2A through 2D) in the instrument.

- **SelectNA™*plus* instrument**:
  The instrument is a contained environment for contamination-free extraction and isolation of microbial DNA. The instrument has a UV source decontaminating the interior surfaces and air. Further, the instrument contains a Pressure Monitoring System (pages 21 to 22) which shall detect residual liquid in case of clogged columns and prevent an overflowing and subsequent contamination of the instrument and other samples. Loading of the instrument with the samples and supplied consumables, including columns, cartridges and enzyme vials, and pipette tips (to be ordered separately; order no. D-925-0xy) should be performed with care to avoid handling-borne contamination. Wear a disposable lab coat, protective gloves, arm sleeves and a disposable mask. Details for loading the instrument are given in the following chapters.

- **Pipette tips**:
  Use only Molzym´s DNA-free *Pipette tips* for the **SelectNA™*plus*** instrument (order no. D-925-0xy).

Part 1: Automated Microbial DNA Isolation

Use the following kits & components:

- ***Kit 1*** *(store at +18 to +25°C)*
  - *ST – Samples tubes* (flip cap tubes) for tissues & swabs pre-treatment only
  - *Plus-SV – Plus-Samples vials* (screw cap vials) for instrument
  - *Extraction columns*
  - *Extraction cartridges*
  - *Buffer cartridges*
  - *ET – Elution tubes*
- ***Necessary components (not supplied with this kit)***
  - *Pipette tips* (Molzym order no. D-925-0xy)
  - *Waste bags* (Molzym order no. D-928-500)

- ***Kit 2*** *(store at -15 to -25°C)*
  - *Enzymes*

Automated Microbial DNA Isolation

Important notes before starting

! Performance of the instrument as specified is guaranteed only if run conditions are followed (**+18 to +25°C**).

! A Class II biological safety cabinet should be near the instrument.

! For equipment, consumables and reagents to be supplied by the user see pages 6 to 6.

! Take care that *MolDNase C, BugLysis plus, Proteinase K* and *Enzyme K* vials (Kit 2) are stored in a freezer (-15 to -25°C) until usage.

Caution: 2-mercaptoethanol is a toxic compound included in *BugLysis plus* vial (yellow capped). Take care not to inhale and otherwise come into contact with when removing the cap.

! Use only fresh samples.
For blood collection, Molzym has evaluated K-EDTA and citrate S-Monovette® (Sarstedt, Germany) for the use with **MolYsis-SelectNA™*plus***. After collection, the sample should be transported to the laboratory and processed immediately. If this is not possible, the sample should be placed in a refrigerator (+4 to +12°C), where it can be stored for 2 days at maximum. ***Do not freeze samples*** to avoid potential loss of microbial DNA due to cell disruption as a result of freezing and thawing. If freezing of samples is desired, use Molzym's *UMD-Tubes* (order no. Z-801-020) which stabilise fluid samples. Thaw samples to room temperature for extraction.

! **Caution**: The following sample materials are inappropriate for the SelectNA™*plus*, because they may clog the *Pipette tips* and the *Extraction columns*:
- Gelatinous samples (e.g., sputum)
- Cell cultures
- Blood cultures with activated carbon
- Swabs on agar gel media
- Samples with transport media including Agar, gel, charcoal medium and Amies

Do not use sample materials of this kind in the instrument to prevent clogging of the *Extraction column* and subsequently rejection of the sample position.

! **Caution**: Wear sterile protective gloves, sterile disposable sleeve covers, a disposable lab coat, protective goggles and a disposable mask when handling infectious material. Work in a Class II biological safety cabinet irradiated with UV before starting according to the instructions of the manufacturer. Follow the instructions of the manufacturer for maintenance of the workstation. Use fresh pipette tips with each pipetting step of the sample preparation (section 1B, page 18).

Sample Collection

Special care has to be taken for sample collection to avoid contamination by skin and environmental microorganisms. It is recommended to transfer the samples to the laboratory for immediate processing (pages 16 to 37). If this is not possible, store the samples in a refrigerator (+4 to +12°C). The stored samples should be analysed within 2 days after sample collection to avoid loss of microbial DNA. For longer storage of fluid samples, Molzym offers *UMD-Tubes* (order no. Z-801-020) which contain a cryoprotectant.

Procedure

Caution: Wear sterile protective gloves, sterile disposable sleeve covers, a disposable lab coat, protective goggles and a disposable mask when handling infectious material. Work in a Class II biological safety cabinet irradiated with UV before starting according to the instructions of the manufacturer. Follow the instructions of the manufacturer for maintenance of the workstation. Use fresh pipette tips with each pipetting step. Do not work under UV irradiation.

1A) How to start

- **Kit 1** contains buffers (in *Buffer cartridges* and bottles) and consumables for the extraction and isolation of DNA from patient samples.

  Open the buffer bottles only in the Class II biological safety cabinet. The Class II biological safety cabinet should stand near the instrument!

  The following used buffer bottles and the unused consumables should be stored at room temperature (+18 to +25°C) in a dark, DNA-free place:

  - Buffers: *SU*, *TSB* and *PKB*
  - *ST tubes* for tissues & swabs pre-treatment only (flip cap tubes, 2.0 ml).
  - *Plus-SV* vials for instrument (screw cap vials).
  - *Extraction columns*
  - *Extraction cartridges*
  - *ET tubes* (*Elution tubes*, flip cap tubes, 1.5 ml)

! Preparation for automated DNA isolation (procedure, section 1C, pages 20 to 32):

For each extraction, use the following tubes:

- For each sample place an *ET* tube (*Elution tube*) in a rack, close the lid and mark the tube with the sample ID.
- For each sample place a *Plus-SV* vial (screw cap) in a rack. Mark the vial with the sample ID. **Caution:** Do not mark the *Plus-SV* vials on the lid, but rather the tubes on the white printed label.
- Only for swabs and enzymatic pre-treatment (tissue and diverse kind of fluid samples, see section 1B, page 18) use the *ST* tube (flip cap). Mark the tubes with the sample ID.

  Caution: Do not use the *ST* tubes in the instrument.

Continue with section 1B, page 18.

- **Kit 2** contains the enzymes.

  Take care that *MolDNase C*, *BugLysis plus, Proteinase K* and *Enzyme K* solutions are stored in a freezer (-15 to -25°C) until usage.

  For each extraction, use a vial each of *MolDNase C*, *BugLysis plus* and *Proteinase K*. **Briefly centrifuge the enzyme vials**, place them in a rack and store the vials in a freezer (-15 to -25°C) for further usage (section 1C, step 9a, page 31).

  For tissue and some kind of fluid samples use *Enzyme K.* Replace the *Enzyme K* vial to the freezer (-15 to -25°C) immediately after handling.

  Caution: Make sure that the enzymes are not frozen when pipetting. Before use, vortex the enzymes and shortly centrifuge the vials to clear the lid.

1B) Preparations for sample loading

For the following sample materials a pre-treatment with *Enzyme K* is essential for the usage in the instrument.

- Fluid samples:
  Mucous fluids, purulent fluids and fluids with flakes of tissue or solid particles (see part *i) Fluid samples*, method 2 of the transfer of the fluid sample).
- Tissue samples:
  Tissues, prosthesis, paraffin blocks, bones and other solid materials (see part *iii) Tissue samples*, page 19).

No enzymatic pre-treatment is necessary for clear or cloudy fluid samples (see part *i) Fluid samples*, method 1 of the transfer of the fluid sample) and swabs (see part *ii) Swabs*, page 19).

Do not use other specimens than validated in Tab. 4, page 13.

i) Fluid samples (ascites, BAL, cerebrospinal, EDTA or citrate-stabilised whole blood, joint aspirates, liver abscess puncture fluid, mucus, plasma, platelet concentrates, pleural fluid, pericardial effusion, synovial fluids, urine)

- Fluid specimens are sampled under aseptic conditions and transported to the laboratory.
- Transfer of the fluid specimens.
  - Method 1: Fluids without enzymatic pre-treatment

    Pipette 1 ml fresh fluid sample from the sample container into a *Plus-SV* vial (screw cap vials, Kit 1). If less sample volume is available, pipette the fluid into a *Plus-SV* vial and fill up to 1 ml (use the measure line of the tube) with buffer *SU* (Kit 1).

    Blood cultures (excluding cultures with activated carbon): Use 0.2 ml of the culture and fill up to 1 ml with buffer *SU*.
  - Method 2: Fluid samples with enzymatic pre-treatment

    Pipette 0.8 ml fresh fluid sample from the sample container into a *ST* tube (flip cap tubes, Kit 1). Add 180 µl of buffer *PKB* (Kit 1) and 20 µl of *Enzyme K* (Kit 2D) in the filled *ST* tube.

    Vortex the *ST* tube at full speed for 15 s and incubate in the thermomixer at 56°C and 1,000 rpm for 10 min.

    After incubation, pipette the fluid phase into a *Plus-SV* vial (screw cap vials, Kit 1) by pipetting. **Avoid transferring any particles that may clog pipette tips and the columns in the instrument.**
    Comment: The particles in the fluid are partially digested and may decay. Potentially present bacteria and fungi are released.
- Transport the rack with the closed Plus-SV vials and *ET* tubes to the instrument. Continue with section 1C; page 20.

Caution: Gelatinous samples (e.g., sputum), cell cultures and blood cultures with activated carbon are inappropriate for the SelectNA™*plus*. This fluid samples may clog pipette tips and the columns in the instrument. Do not use these sample materials with the instrument.

ii) Swabs (bones, mouth, nasopharynx, wounds)

- Do use only swabs without agar gel
- Pipette 1 ml of buffer *SU* (Kit 1) into a *ST* tube (flip cap tubes, Kit 1). If there is fluid in the swab vial, pipette 1 ml thereof into a *ST* tube instead of buffer *SU*. In case of less sample volume available, fill up to 1 ml by pipetting buffer *SU* to the sample in the *ST* tube (use the measure line of the tube).
- Remove the swab from the swab vial and transfer to the *ST* tube.
- Wash the swab by swirling in the fluid and pressing to the wall of the *ST* tube several times. Thereafter discard the swab.
- Transfer the sample from the *ST* tube into a *Plus-SV* vial (screw cap vial, Kit 1). Transport the rack with the closed *Plus-SV* vials and *ET* tubes to the instrument. Continue with section 1C; page 20.

Caution: Swabs on agar gel media are inappropriate for the SelectNA™*plus*. This material in the swabs may clog pipette tips and the columns in the instrument. Do not use these sample materials with the instrument.

iii) Tissue samples (abscesses, aorta, artificial tissues, biopsies, bone marrow heart valves, lung tissues, mucous, pacemakers, paraffin blocks, pericard prosthesis)

- Tissue specimens are sampled under aseptic conditions and transported to the laboratory.
- Pipette 180 µl of buffer *PKB* (Kit 1) into a *ST* tube (flip cap tubes, Kit 1).
- Transfer the specimen to a sterile support, e.g., a Petri dish, by using sterile forceps. For preparation of the tissue specimen, the area should measure at maximum approx. 0.5 x 0.5 x 0.5 cm. Cut the specimen into small pieces by using a sterile scalpel or sterile preparation scissors. Thereafter, transfer the dissected specimen to the *ST tube* filled with buffer *PKB*. The specimen should be covered completely by the buffer. Add 20 µl of *Enzyme K* (Kit 2D) to the specimen.
- Vortex the *ST tube* at full speed for 15 s and incubate in the thermomixer at 56°C and 1,000 rpm for 10 min.
  Comment: The tissue is partially digested and may decay. Potentially present bacteria and fungi are released.
- After incubation, pipette the fluid phase into a *Plus-SV* vial (screw cap vials, Kit 1) by pipetting.

  ! For this use the 200 µl pipette. **Avoid transferring any particles that may clog pipette tips and the columns in the instrument.**
- Fill up to 1 ml with the transport solution (**avoid transferring any tissue particles** from the transport solution), if available, or with buffer *TSB* (use the measure line of the tube). Transport the rack with the closed *Plus-SV* vials and *ET* tubes to the instrument. Continue with section 1C; page 20.

1C) Instrument Setup

Pathogen DNA is extracted and purified from clinical fluid samples, swabs and tissues in the ***SelectNA™plus*** instrument.

The Instrument

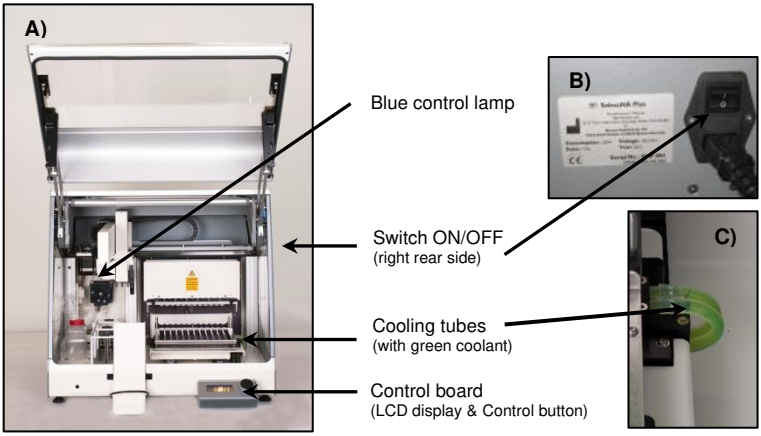

Fig. 2: The **SelectNA™*plus*** instrument.

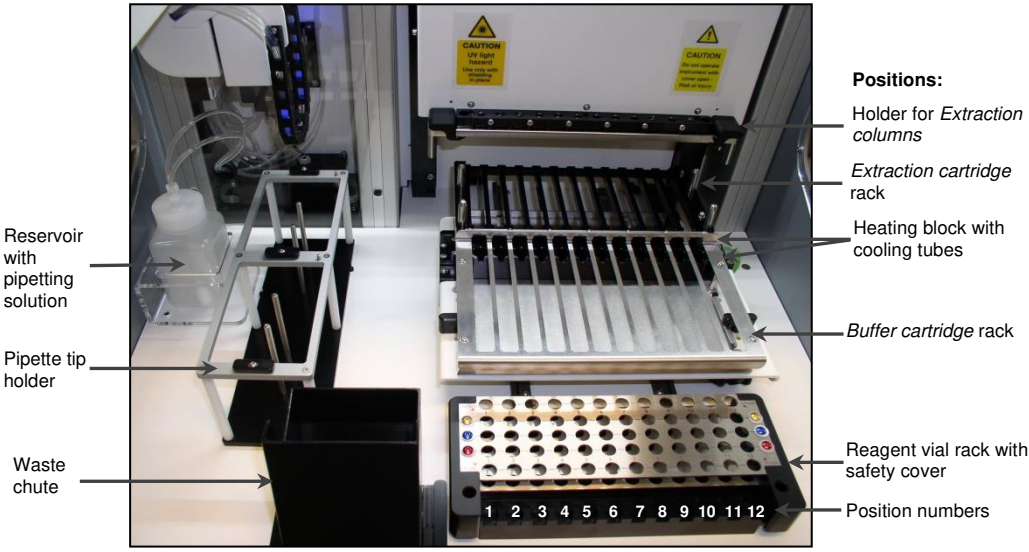

Fig. 3: The interior of the **SelectNA™*plus*** instrument.

Pressure Monitoring System

The ***SelectNA™plus*** instrument includes a pressure monitoring system to reduce the risk of overflowing *Extraction columns*.

The system controls the *Extraction columns* after the filtration steps of the lysate. If residual liquid is detected on the column, the position is switched off and fluid is no longer transferred to this column position. The switched off positions will be indicated at the end of the extraction program in the display as “rejected channel” (details on page 22).

The pipetting arm is equipped with a sensor box including the pressure monitoring system (Fig. 4). The ***SelectNA™plus*** instrument possesses a four-channel pipette tip picking head for the uptake of up to four pipette tips at a time. For each channel a blue LED is located on the sensor box (Fig. 4).

Fig. 4: Pipetting arm with new pressure monitoring system.

Fig. 5: Four channels of the pressure monitoring system. The blue LED is switched on if the corresponding sample position of the pipetting block is blocked.

The blue LED is switched on if the corresponding column position is blocked or positions are not used during the pipetting process (e.g., 2 samples processed and LEDs of channels 3 and 4 switched on). The LED signals change for the next pipetting block. First pipetting block with samples 1 to 4, second block with samples 5 to 8 and third block with samples 9 to 12.

For example: Channel 1 is switched on in the first block (Fig. 5, part A). The column on sample position 1 is rejected. At the next block (samples 5 to 8) channel 3 is switched on (Fig. 5, part C) and the sample position 7 is rejected.

When the extraction program is finished, any rejected sample positions will be shown in the display, when applicable. It shows `Rejected channel: (position number)` and every position needs to be confirmed by pressing the `Control button` before the next position is displayed.

Example: Channel 3 is switched on in the first pipetting block and channel 4 in the third block. In the end it is summarized in the display with position 3 (Fig. 6, part A) and after confirming with the `Control button` with position 12 (Fig. 6, part B).

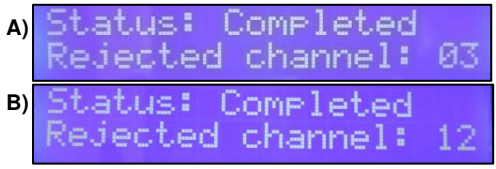

Fig. 6: Display shows the rejected positions at the end of the program. Example: A) The first rejected position 3 and B) the second rejected positon 12.

Loading Procedure of Components

Use Kits 1 (Cartridges & Consumables) and 2 (Enzymes).

Caution: Load the components direct in the instrument. Wear sterile protective gloves, sterile disposable sleeve covers, a disposable lab coat, protective goggles and a disposable mask when working in the instrument, loading the components and removing the *Elution tubes* after the extraction run (steps 2, 5 to 9, pages 24 to 35).

1. Initialisation and selection of the protocol

Fig. 7: Control board with Control button of the instrument.

- Turn ON the instrument on the right rear side of the instrument (Fig. 2B, page 20). The blue control lamp inside the instrument is on (Fig. 2, part A, page 20).
- Press the 'Control button' (Fig. 7) of the front control board to initialise the instrument.
- Press the 'Control button' to select the protocol from the 'Run Program Menu'.
- Turn 'Control button' to select script no. 1 'SelectNAplus' and press the button.

2. Waste bag

Place a *Waste bag* (not supplied, order no. D-928-500) to the waste chute and fix with the rubber ring (Fig. 8).

Caution: The exit of the waste chute must not be blocked by the waste bag, because otherwise pipette tips may accumulate in the chute and may fall into the interior of the instrument.

The screw caps of the enzyme and *Plus-SV* vials (step 9a and 9c, page 31) could be disposed to the waste bag.

Fig. 8: Waste chute with fixed *Waste bag*.

Load the following components (steps 3 and 5 to 9) direct in the instrument.

3. Reservoir with pipetting solution

Check that the reservoir is completely filled with pipetting solution (250 ml autoclaved deionized water;Fig. 9, on the left, and Fig. 3, page 20).

The tubings must be arranged on the left side of the reservoir (Fig. 9), because otherwise the tubes may block the pipette tip holder.

Confirm the loading step 'Load DI water bottle (250 ml DI water)' with the 'Control button'.

Fig. 9: The reservoir with pipetting solution.

4. Selection of the number of samples

- Select the number of samples to be processed. For this, turn the 'Control button' clockwise to select. The display shows the required number of full pipette tip rows, that are needed for the number of samples intended to be extracted. For further information on the tip rows see point 4 `Pipette tips`.
- Confirm 'Yes' and press the `Control button`. Select `No` to correct the number of samples. By selecting `No` you come back to the `Main Menu`.

5. Pipette tips

Note: Pipette tips for the instrument are not supplied with this kit. Use only Molzym´s DNA-free *Pipette tips* (order no. D-925-0xy) to avoid DNA contamination.

The pipette tip holder is loaded with two pipette tip racks. Each tip rack contains 96 tips that are arranged in 12 rows with 8 tips each (A1 to H1; see Fig. 10). The programming of the instrument follows an algorithm that is optimised for the usage of full tip rows remaining in the pipette tip holder after an extraction run. Tab. 5 shows the consumption of pipette tip rows for the needed number of samples. An example on page 26 is presented to explain the algorithm.

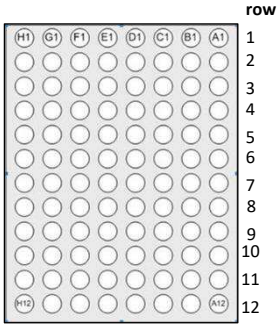

Fig. 10: Pipette tip rack

Tab. 5: Consumption of pipette tip rows dependent on the samples processed.

No. of samples	Tip rows used
1	4
2	8
3	8
4	8
5	10
6	12
7	16
8	16
9	18
10	20
11	23
12	23

Loading of the pipette tip holder:

Carefully load the pipette tip holder (Fig. 11, part 1) with pipette tip racks in the correct direction. Avoid touching the pipette tips on the tip holder. Fix the racks with the three black clips of the holder (Fig. 11, part 2 and 3).

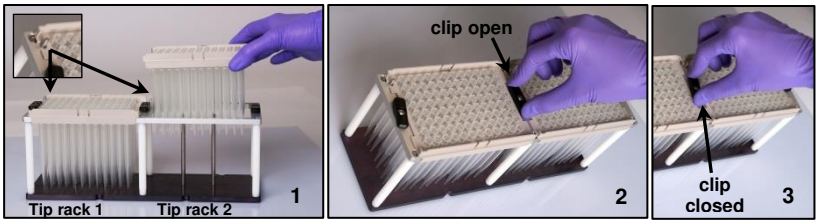

Fig. 11: Loading procedure of pipette tip racks into the tip holder.

Place the loaded pipette tip holder into the instrument (Fig. 12). Fit the notches of the holder in the 4 black knobs at the bottom of the instrument (Fig. 12, part 2 and 3).

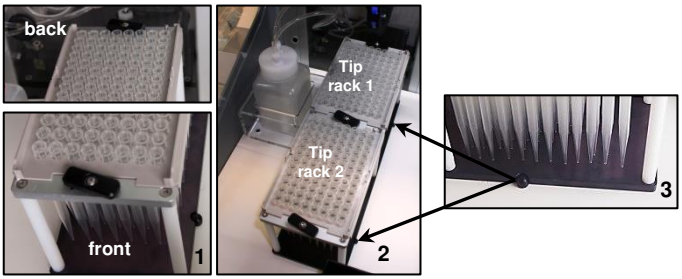

Fig. 12: Loading *Pipette tips* into the instrument.

Be sure that there are enough filled tip rows to run the selected number of samples (Tab. 5, page 24). The display shows the required number of rows. Select the tip row position (A1 to A12) and the tip rack (1 or 2). For the selection turn the ‘Control button’ clockwise.
Confirm this loading steps ‘Load Pipette tip racks’ and ‘enter starting at full tip row’ with ‘Control button’.
Confirm `Yes` for the selected `starting tip full row` with the button.
Select ‘No’ to return to section 4 `Selection of the number of samples` (page 24).

Explanation of the algorithm for usage the pipette tip rows:

In this example, the instrument’s pipette tip holder was initially loaded with two full pipette tip racks (2x 96 tips; see Fig. 12) and run with 7 samples which consumed 16 tip rows (Tab. 5, page 24). Accordingly, the tip rack 1 is empty and the tip rack 2 has 8 left over rows completely filled with tips (Fig. 13, page 26; tip rack 2 position row A5 to A12).

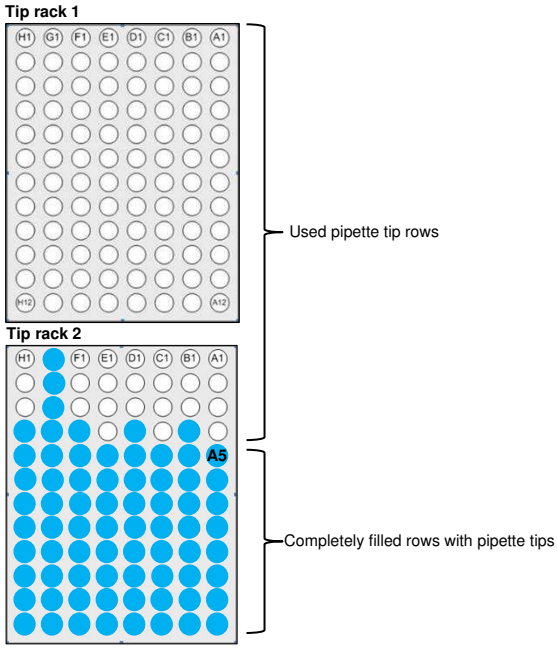

Fig. 13: Pipette tip racks at the end of running 7 samples. White positions are empty, blue positions filled with pipette tips.

Reference to Tab. 5 (page 24) tells one that up to 4 samples can be processed in a following run. Continuing with this example, if more than 4 samples (up to 10 samples) are desired to be processed, the following is recommended. Take care to avoid handling-borne contamination. Wear a disposable lab coat, protective gloves, arm sleeves and a disposable mask and follow the advices for avoidance of contamination (page 14).

Remove the empty tip rack 1 and place the partially filled tip rack 2 (Fig. 13) to the position of rack 1. Then place a new, full tip rack to tip rack position 2. Now, 8 rows (tip rack 1, position A5 to A12) plus 12 rows (tip rack 2), in total 20 rows are available to run up to 10 samples (Tab. 5, page 24). Continue loading the instrument with the other consumables and reagents and the sample as below.

For process 11 to 12 samples use two new full tip racks.

6. *Extraction columns*

Pick the columns with a sterile forceps and place them into the column rack from the left to the right side (Fig. 14).

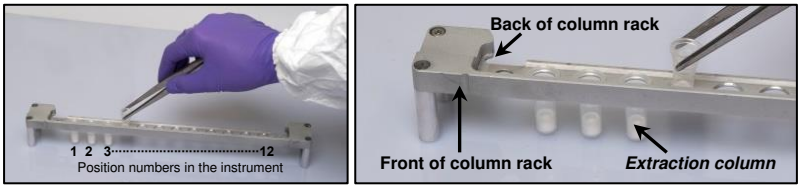

Fig. 14: Loading procedure of *Extraction columns* in the column rack.

Place the filled rack into the instrument in the black holder of the column rack. Push the column rack completely into the holder (Fig. 15, part 1).
Fix the rack with the black clips on each side of the holder (Fig. 15, parts 2 and 3).
Confirm this loading step ‘Load Extraction columns’ with ‘Control button’.

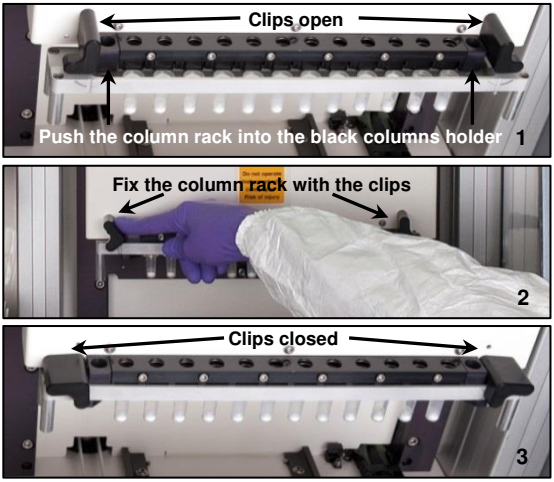

Fig. 15: Loading procedure of the filled column rack into the instrument.

7. *Extraction cartridges*

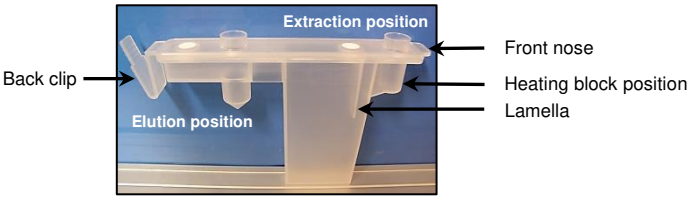

Fig. 16: *Extraction cartridge*

Load the extraction rack with the *Extraction cartridges* (Fig. 16), starting from the left to the right side (position numbers 1 to 12, Fig. 17, part 1).
Place the front nose of the *Extraction cartridge* in a slanted angle under the metal edge (Fig. 17, part 1).
Push the *Extraction cartridge* into the rack until the back clip locks in position with a click sound (Fig. 17, part 2).

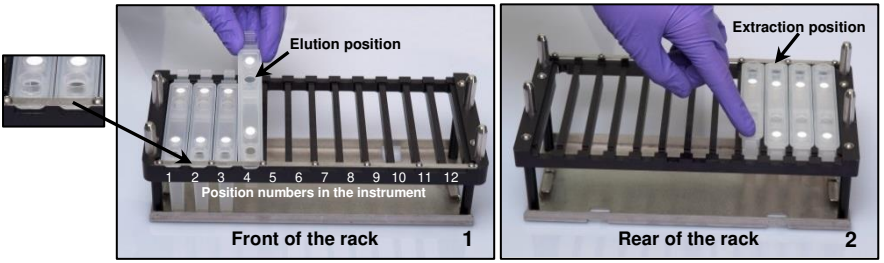

Fig. 17: Loading procedure of *Extraction cartridges* into the extraction rack.

Place the rack into the instrument. Take care that cartridges are placed with the extraction position in the corresponding indentation in the heating block. Fix the rack with the black clips on both sides (Fig. 18).
Confirm this loading step ‘Load vacuum cartridges’ with ‘Control button’.

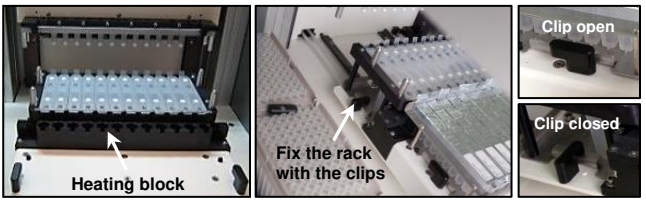

Fig. 18: Loading procedure of the filled *Extraction cartridge* rack into the instrument.

8. *Buffer cartridges*

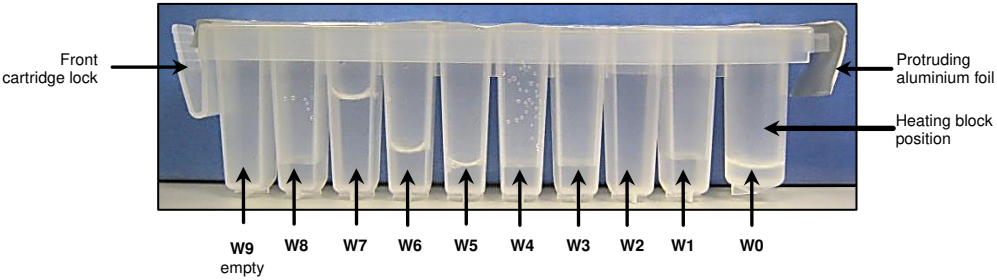

Fig. 19: Filled Buffer cartridge (DNA-free).

Bend up the protruding aluminium foil at the rear of the *Buffer cartridge* and load the buffer rack with the *Buffer cartridges* (Fig. 19). Place the front nose of the *Buffer cartridge* in a slanted angle under the metal edge (Fig. 20, part 1). Push the *Buffer cartridge* in the rack until the back clip locks in position with a click sound (Fig. 20, part 2).

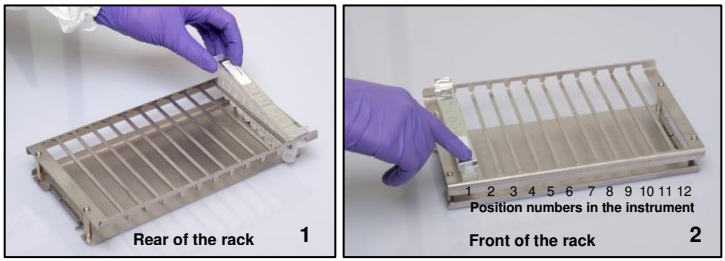

Fig. 20: Loading procedure of *Buffer cartridges* into the rack.

Place the rack into the instrument. Take care that the back position of the cartridge (round W0) is placed into the corresponding indentation in the heating block (Fig. 21). Fix the rack with the black clips on both sides (Fig. 21).

Fig. 21: Loading procedure of the filled buffer rack into the instrument.

Carefully peel off the aluminium foil by pulling constantly and slightly directed to the side (Fig. 22, parts 1 and 2). Do not touch the reagent wells. At the end, check that the cartridges are fixed flat in the rack.
Confirm this loading step ‘Load Buffer cartridges and peel off aluminium foil’ with the ‘Control button’.

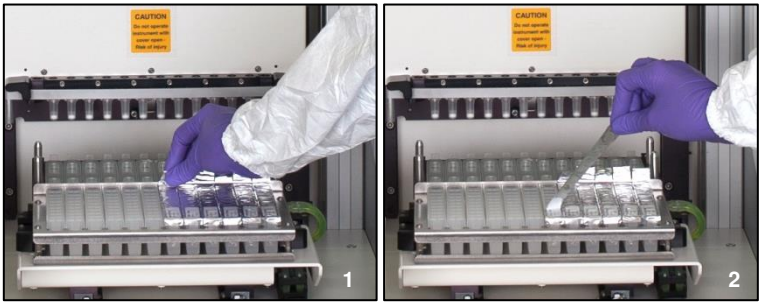

Fig. 22: Removing of the aluminium foil of the *Buffer cartridges*.

9. Loading of Reagent vial rack

Close the door and press ‘Control button’ for loading the Reagent vial rack.

The display on the Control board reads:
‘**WARNING** Transferring rack. Press button’

Press the button again and the rack with the cartridges moves backward. **Caution:** Keep your hands off the instrument!

After movement of the cartridge rack, open the door. Place the Reagent vial rack into the instrument.

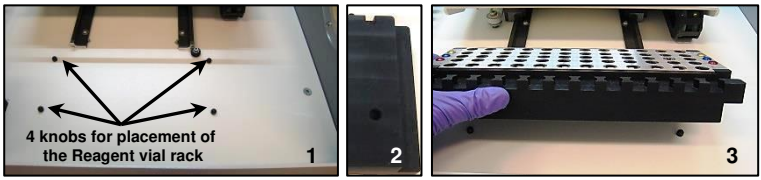

Fig. 23: Placing Reagent vial rack in the instrument.

For this, place the notches on the bottom of the rack (Fig. 23, part 2) into the 4 black knobs at the bottom of the instrument (Fig. 23, part 1). For this, slide the rear bottom notches of the rack in the rear knobs (Fig. 23, part 3) and then place the front bottom notches in the corresponding knobs.

Place the following vials in the sequential steps (parts 9a to 9c, next page) in the Reagent vial rack of the instrument (Fig. 24, page 31). Place the vials from left to right. Each row of enzyme vials is marked with a coloured knob at the left and right side of the Reagent vial rack.

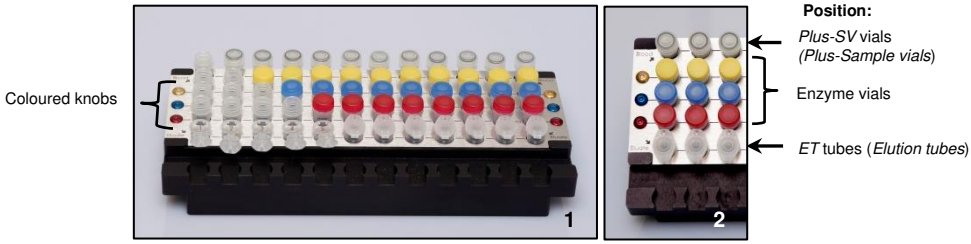

Fig. 24: Reagent vial rack

9a) Enzymes and colour code

- *BugLysis plus*, yellow cap
- *Proteinase K*, blue cap
- *MolDNase C*, red cap

! Remove the rack with enzyme vials from the freezer (section 1A, page 17).
Make sure that the enzyme vials have been pulse centrifuged to clear the lids.

At first, open the *BugLysis plus* vials (yellow cap) and place them into the Reagent vial rack at the position in row with the yellow coloured knob, following positions 1 to 12 in the Reagent vial rack from left to right (Fig. 24). Dispose the screw caps to the waste bag.

Caution: *BugLysis plus* contains 2-mercaptoethanol which is toxic. Take care not to inhale and otherwise come into contact with.

Secondly, open the *Proteinase K* vials (blue cap) and place them into the Reagent vial rack at the position in row with the blue coloured knob, following positions 1 to 12 in the Reagent vial rack from left to right (Fig. 24). Dispose the screw caps to the waste bag. Confirm the loading step 'Load reagents rack, Enzymes blue cap' with 'Control button'.

Lastly, open the *MolDNase C* vials (red cap) and place them into the Reagent vial rack at the position in row with the red coloured knob, following positions 1 to 12 in the Reagent vial rack from left to right (Fig. 24). Dispose the screw caps to the waste bag.

9b) *Elution tubes* (*ET* tubes)

Open lids of the Elution tubes, marked with the sample ID (section A, page 17). Place the opened *Elution tubes* to the elution position following positions no. 1 to12 of the Reagent vial rack (left to right; Fig. 24). Adjust the lids of the tubes to the front of the rack (part 1; Fig. 24).

9c) *Plus-Sample vials* (*Plus-SV* vials)

Remove the screw cap from each *Plus-SV* vial (see part B, page 18) containing the sample and place into the Reagent vial rack following positions 1 to 12 (left to right; Fig. 24). Dispose the screw caps to the waste bag.

9d) *Safety cover*

After loading of the Reagent vial rack with enzymes, *Elution tubes* and the *Plus-SV* vials place the Safety cover on the rack, if available for the instrument (Fig. 25).

Fig. 25: Safety cover for the Reagent vial rack.

Confirm the loading step ‘Load reagents rack ` with ‘Control button’.

Check that the aluminium foils of the *Buffer cartridges* have been removed.

Check, that all vials stand correctly at the same level in the rack and all caps have been removed. Confirm this step `Check all caps removed` with the ‘Control button’.

Close the door of the instrument.

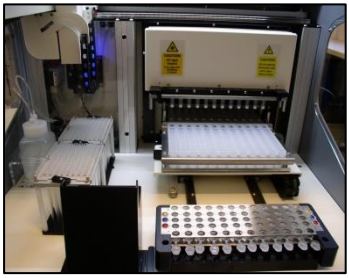

Fig. 26: Loaded instrument.

10. Waste chute and bag

Check that a waste bag was fixed at the waste chute and the exit of the chute is not blocked with the bag. Otherwise pipette tips may accumulate in the chute and may fall into the interior of the instrument. Further information see step 2, page 23.

Confirm the loading step ‘Load waste chute and bag’ with the ‘Control button’.

11. Start the extraction process

Close the door of the instrument and confirm this step 'Close door and press button to start' with 'Control button'. The instrument now starts the extraction.

The program is finished with a signal sound. The approx. total time of the program for the corresponding sample number see in Tab. 6.

Tab. 6: Total time of the extraction process.

No. of samples			Approx. total time [min]
1	to	4	95
5	to	8	108
9	to	12	126

Caution: Wear sterile protective gloves, sterile disposable sleeve covers, a disposable lab coat, protective goggles and a disposable mask when opening the door for closing and removing the *Elution tubes* from the instrument.

Open the door and remove the Safety cover of the Reagent vial rack (if available). Close the *Elution tubes* including the eluate and remove them from the instrument. Confirm this step 'Open door' with the 'Control button'.

Any samples rejected by the Pressure Monitoring System (pages 21 to 22) will be shown in the display, when applicable. Every rejected position needs to be confirmed by pressing the 'Control button'.

12. Eluted DNA

Store the eluted DNA at +4 to +12°C if analysed within 48 hours or freeze at -15 to -25°C for longer storage. Avoid frequent freeze-thaw cycles, because this may result in loss of eluted DNA (in particular at low DNA concentrations).

<u>Volume of the eluate:</u>
The mean eluate volume amounts to 90 µl (range approx. 70 to 140 µl). A volume <70 µl is possible and does not have influence on the result.
If volumes are higher, this may indicate a malfunction of the drying of the membrane which leads to elution of ethanol. This in turn would inhibit the PCR reaction. In this case the extraction should be repeated.

Part 2: Cleaning Process of the Instrument

Use the following components:

- ***Supplied with the SelectNA™plus instrument***
  - *4 Cleaning Cartridges (can be ordered under Molzym order no. D-927-012)*

- ***Components not supplied***
  - *Low-lint soft paper towel.*
  - *Disinfectant e.g., Meliseptol® (Braun, Germany) or 70 % (v/v) ethanol.*
  - *1% bleach solution (active Cl_2; sodium hypochlorite).*

Decontamination of the Instrument

You need a low-lint, soft paper towel for the cleaning procedure. For disinfection employ Meliseptol® New Formula (B. Braun, Germany) or an ethanol containing disinfectant. After use, dispose the paper towel in the waste for infectious material. Do not spray surfaces inside the instrument with the disinfectant. Instead use a paper towel sprayed with disinfectant and wipe the surfaces.

2A) Decontamination after each run

1. Dispose the empty *Plus-SV vials* and enzyme vials to the waste bag (use the waste chute). **Caution**: 2-mercaptoethanol is a toxic compound included in *BugLysis plus* vial (yellow capped). Take care not to inhale and otherwise come into contact with when removing the vial.
2. Decontamination of the waste chute: Spray the contaminated surfaces with the disinfectant and wait for 10 min.
3. Remove the Reagent vial rack. Clean the rack and his Safety cover. Wipe the surface of the instrument around the rack with a paper towel soaked with disinfectant. Replace the rack back to the instrument.
4. The display of the Control board indicates: '**WARNING** Transferring rack. Press button'. Close the door.

   Press the button and the vacuum rack with the cartridges transfers forward. **Caution:** Keep your hands off the instrument!
5. Remove the waste bag and dispose the used *Buffer cartridges*, *Extraction cartridges* and *Extraction columns* to the waste bag. For this, open the clips of the racks and take out the racks with the used consumables. At the end, dispose the waste bag to the waste for infectious material.

   Caution: Do not take out single cartridges (*Buffer* and *Extraction cartridges*) from the racks while the racks are fixed in the instrument. This may damage the racks and heating blocks.

   Note: In case of a contaminated filter of the *Extraction cartridge* (extraction position) with liquid from the waste, clean the corresponding suction cup of the vacuum system. For cleaning see section 1F, page 37.
6. Storage partially filled tip racks in the pipette tip holder in the instrument. The completely filled tip rows in rack 1 or 2 can be used for the following run. Remove empty tip racks from the pipette tip holder.
   If applicable, remove the tip rack 1 from the pipette tip holder. Place the partially filled tip rack 2 to position of rack 1 of the pipette tip holder.

   Note: Take care to avoid handling-borne contamination and follow the advices for avoidance of contamination (manual on page 14).
   Do not use the remaining pipette tips in the used rows and do not re-assemble the tips in the pipetting tip racks to avoid contamination of following extractions.
7. Clean the removed racks. For this wet the racks completely with disinfectant and wait for 5 min. After the time of exposure wipe the racks with a paper towel soaked with the disinfectant and let dry the racks on air before replace into the instrument.
   Clean the suction cups and the surfaces of the instrument with a paper towel soaked with disinfectant.

   Do not spray the interior of the instrument! **Caution:** Do not clean the pipetting arm, the control board, the chains, cooling tubes and the windows of the instrument with the disinfectant.

8. Replace all racks to the instrument. Place the column rack to the right side of the pipette tip holder for UV cleaning (Fig. 27, part 1).

   Place the cleaned safety cover of the reagent vial rack on the right side of the instrument. The back side of the cover is face in the inside of the instrument (Fig. 27, part 1 and 2). The cover is place on the black feet (Fig. 27, part 2).

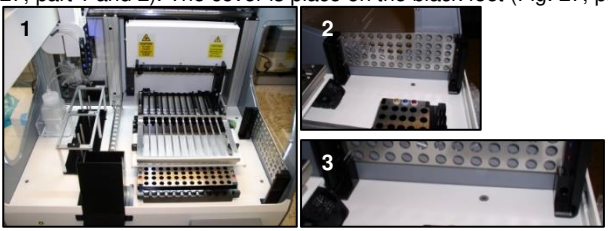

Fig. 27: Interior of the instrument for UV decontamination of the column rack and safety cover.

9. Check that minimum 100 ml pipetting solution (autoclaved deionized water) is still in the reservoir. If not, fill the reservoir completely (max. 250 ml) with autoclaved deionised water (Fig. 3, page 20).

   Press the 'Control button' to finalise the protocol.

10. Close the door. Wipe the door handle and the door top with a paper towel soaked with disinfectant and select the 'UV decontamination' from the 'Main Menu'. Confirm the program with the 'Control button'. Confirm the loading steps of the empty racks with the 'Control button'. The instrument now starts the UV decontamination. The program is finished with a signal sound. Open the door and confirm this with the 'Control button'.

    ! Decontamination (steps 1 to 6, section 1D) and UV decontamination should be performed after each run of the instrument. The instrument must be empty before starting the decontamination program.

11. Start a new extraction run or continue with section 1E 'Daily decontamination of the instrument'.

2B) Daily decontamination of the Instrument

At the end of each day of usage clean the waste chute.

1. Remove the decontaminated waste chute (section 1D 'Decontamination of the instrument after every extraction run') from the instrument and clean it.
2. Clean the chute in a special washing machine using a mildly alkaline cleaning powder with sodium hydroxide (e.g. LABWASH® Premium Classic, VWR). Dry the clean chute with a low-lint, soft paper towel.

   Optionally, put the waste chute in a washing solution (e.g., LABWASH® Premium Classic, VWR). The waste chute must be completely covered by the washing solution. Incubate the waste chute as described in the instruction and rinse the chute with water. Dry it with a low-lint, soft paper towel.
3. Thereafter place the clean chute back to the instrument
4. Close the door and select script 2 'UV decontamination' from the 'Run Program Menu'. Confirm the step 'Start decontamination' with the 'Control button'. The instrument now starts the UV decontamination. After 5 min the program is finished with signal sounds. Confirm this with the 'Control button'.

   ! The instrument must be empty before starting the decontamination program.

2C) Cleaning script - Cleaning of the pipetting system

Clean the pipetting system every 14 days of usage.

1. Supplied material to be used: 4 *Cleaning cartridges* (Fig. 28; supplied with the instrument, Molzym order no. D-927-012) and *Cleaning bottle* (Fig. 28).

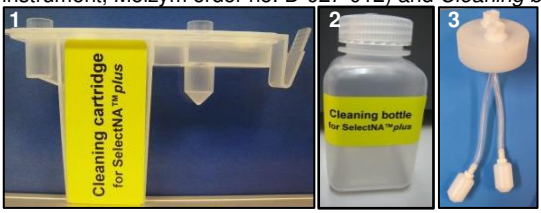

Fig. 28: *Cleaning cartridge* (1), *Cleaning bottle* (2) and lid (3).

Prepare a bleach solution (1 % (active Cl_2) sodium hypochlorite): For this, mix 14.3 ml of sodium hypochlorite (14 % active Cl_2, VWR Chemicals) and 185.7 ml autoclaved deionized water. Fill the *Cleaning bottle* with 100 ml prepared bleach solution.

2. Start the cleaning script from the 'Cleaning Menu' for cleaning the pipetting tubes.
3. Load the four *Cleaning cartridges* (Fig. 28, page 37) into the rack of the *Extraction cartridges* (positions 1 to 4), place the rack into the instrument and fix on both sides with the black clips (see Fig. 17 and Fig. 18, page 28). Confirm the step with the 'Control button'.
4. Remove the reservoir with the pipetting solution incl. the lid (Fig. 9, page 23) from the instrument. The tubing are fixed with a click-system on the lid. Connect the filled *Cleaning bottle* with the second lid for the pipetting tubes. Confirm the step 'Load cleaning bottle (100 ml 1 % bleach)' with the 'Control button'.
5. Close the door of the instrument and start the cleaning procedure by pressing the 'Control button'. The tubing incubates 10 min with the 1 % bleach solution.
6. Empty the reservoir bottle and clean with 1 % bleach. For this, fill the bottle with the bleach solution (~50 ml), close it with the screw cap of the *Cleaning bottle* and shake it. Empty the bottle and rinse with autoclaved deionized water.

   Clean the lid of the reservoir bottle with a low-lint, soft paper towel soaked with the 1 % bleach solution. After this, rinse the lid with autoclaved deionized water and dry it with a paper towel or on air. Fill reservoir with 250 ml autoclaved deionized water and close it with the cleaned lid.
7. After the 'soaking (wash)' step (10 min) indicates with a signal sound, open the door and remove the *Cleaning bottle* incl. the lid. Connect the cleaned reservoir filled with autoclaved water on the tubing. Close the door of the instrument and resume the program by pressing the 'Control button' to rinse the pipetting tubes.
8. Remove the lid from the *Cleaning bottle*, close it with the screw cap and shake it. Empty the bottle and rinse with autoclaved deionized water. Clean the lid (with the tubing) of the *Cleaning bottle* as per description of the lid in step 6.
9. At the end of the cleaning program, remove the cartridge rack from the instrument. **Caution:** Do not take out the *Cleaning cartridges* from the rack while fixed in the instrument. This may damage the racks and heating block.
10. Dispose the solution from the *Cleaning cartridges* and rinse with water. Dry the *Cleaning cartridges* on air and use for the next cleaning procedure.

11. Clean the cartridge rack with a paper towel soaked with disinfectant. Replace the empty rack into the instrument.
12. Close the door of the instrument, select the 'UV decontamination' (Version V.0.04) from the 'Main Menu' and start the UV decontamination program. After 5 min the program is finished indicated by a signal sound. Shortly lift the door to close the signal sound.

Caution: Dispose waste including sodium hypochlorite in accordance with federal, state and local regulations. Avoid runoff into storm sewers and ditches which lead to waterways (concentration active chlorine >0.25 %).

Dispose bleach solution separately to the *Extraction cartridges* and Enzyme vials, because of risk of cyanide formation.

2D) Cleaning of the vacuum system

In a case of an overflowed sample clean the white suction cups of the vacuum system, the black holder of the column rack (Fig. 29) and the column rack by wiping. Do not spray the interior of the instrument!

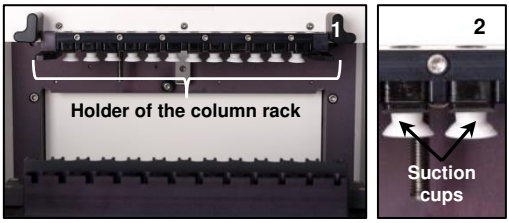

Fig. 29: Holder of the column rack (1) and suction cups (2) of the vacuum system.

For this, pull the suction cups from the system and clean with a paper towel soaked with disinfectant (Fig. 30). Push the dry suction cups back to their position in the column holder.

Fig. 30: Cleaning of the suction cups.

For further information see `Troubleshooting` on page 41.

Supplementary Information

Troubleshooting

This guide may help solve problems that may arise. For further support:
Phone: +49(0)421 69 61 62 0 • **E-Mail:** support@molzym.com

Observation	Possible cause	Comments/suggestions
Strong human DNA background in gel electrophoresis or Real-Time PCR	• Enzymes not used in the correct order.	Ensure that all enzymes are placed in the correct position in the reagents vial rack (coloured code).
	• Enzymes volume too low.	Ensure that all enzyme vials are briefly centrifuged before use. Make sure that the enzymes are not frozen if placed in the instrument
No pathogen DNA detectable in spiking tests with *SU* buffer	• Insufficient lysis	Ensure that all enzymes are placed in the correct position in the reagents vial rack (coloured code). Ensure that all enzyme vials are briefly centrifuged before use.
	• PCR inhibition	The *Extraction column* was clogged and inhibitors are co-eluted. The filter of the *Extraction cartridge* was moist/contaminated and the eluate volume higher than normal. Inhibitors like ethanol are co-eluted.
	• Pathogen load too low (below limit of detection)	Check the load of the pathogen by plating and increase the titre for inoculation.
	• Loss of nucleic acids during the storage of the eluate.	Store the eluted DNA at +4 to +12°C if analysed within 48h or freeze at -15 to -25°C until further use. Avoid frequent freeze-thaw cycles, because this may result in a decay of the eluted DNA (in particular at low DNA concentrations).
False positive PCR result (signal in negative PCR control)	• Cross contamination • Contamination during handling.	Principally, work under UV-irradiated workstations. Avoid the generation of aerosols by careful pipetting the samples and buffers. Open buffer bottles only shortly for pipetting and close again immediately thereafter. Frequently change gloves. UV-irradiate the workstation at the end of handling a series of samples. Prepare mastermixes and handle DNA samples under different UV workstations (page 14). Clean the vacuum system (section 1F, page 37). Run cleaning program at least once a week (section 1G, pages 38).
	• Contaminated *Pipette tips*	Use **only** Molzym´s DNA-free Pipette tips (order no. D-925-0xy) to avoid DNA contamination. Do not use the remaining pipette tips in the used tip rows and do **not re-assemble** the tips in the pipetting tip racks to avoid contamination of following extractions

Observation	Possible cause	Comments/suggestions
False negative PCR result	• PCR inhibitors co-eluted	Ensure that all enzymes are placed in the correct position in the reagents vial rack (coloured code). Ensure that all enzyme vials are briefly centrifuged before use. Make sure that the eluate volume is in the range 70 to 140 µl. If higher, the column was clogged which leads to the co-elution of ethanol. Ethanol is a strong PCR inhibitor. In this case the extraction should be repeated. A volume <70 µl is possible and does not have an influence on the result.
No eluate	• Process error; elution buffer on the column.	Place the column into the *ET* tube, close the lid and briefly centrifuge the tube. **Caution:** Avoid contamination by handling. Wear sterile disposables (gloves, sleeve covers, lab coat), a disposable mask and protective goggles. The result of the sample analysis may deviate from the evaluated extraction process.
Eluate volume <70 µl	• The column was partial clogged with remaining particles of the sample (e.g., tissue).	Check carefully the Extraction Control Assay (MA Control) and if needed repeat the extraction.
Eluate volume >140 µl	• Malfunction of the drying of the column.	Check carefully the Extraction Control Assay (MA Control) and if needed repeat the extraction.
Error messages	• Error message indicated by an alarm sound when starting the extraction script.	The control board shows the following error code: 028-007-004-000 013-051-000-000 000-000-000-000 Switch off the instrument and start the extraction scrip once again. If the problem with the script persists, contact the technical support for help.
	• Error message during operation.	Switch off the instrument and start it again. Select the 'Service Menu' and choose 'Display Error Log'. Note the error code. Contact the technical support for help.

Observation	Possible cause	Comments/suggestions
Incorrect picking of Pipette tips in the instrument	• Rejected tips in the racks, no tip picking on positions	Depending on the number of samples, it is normal for pipette tips to remain in some positions in the racks. Check if this is the case, otherwise contact the technical support for help. Remaining Pipette tip (orientation for position coordinates see Fig. 10, page 24)

Sample No.	**rows** (excluding full rows)		**positions**
1	1-4	rack 1	H, F, D, B
2	1-8	rack 1	H, F, D, B
3	1-8	rack 1	G
4	only full rows		
5	9	rack 1	G
6	12	rack 1	G, E
7	2-12 1-3 4	rack 1 rack 2 rack 2	G G H, G, F, D, B,
8	4	rack 2	G, E, C, A
9	4 6	rack 2 rack 2	G G, E, C, A
10	8	rack 2	H, G, E, F, D, B
11	2,4,5, 8,11 1,2,4, 5,7,8, 10.11	rack 1 rack 2	G G
12	only full rows		

Rejected sample positions	• The column was clogged and the position switched off during the extraction process	**Cause**: Incomplete solubilisation of the sample. **Fluid samples**: - Use only freshly collected samples or samples stored at +4 to +12°C for no longer than 2 days. For longer storage of fluid samples use Molzym´s *UMD-Tubes* (order no. Z-801-020) which contain a cryoprotectant. - Sputum and cell culture are inappropriate for the SelectNA™*plus*. These fluid samples may clog pipette tips and the column in the instrument. - Use for mucous fluids, purulent fluids and fluids with flakes of tissue or solid particles an enzymatic pre-treatment step. Ensure that samples do not contain particles after digestion (section 1B, pages 18 to 19). These samples may clog pipette tips and the column in the instrument without the pre-treatment. **Tissue samples:** - Ensure that samples do not contain particles after digestion (section 1B, pages 18 to 19).

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Tradenames

Tradename	Factory
Biosphere® plus	Sarstedt
GuardOne® Werkbank	Starlab
LABWASH® Premium Classic	VWR Chemicals
Meliseptol® New Formula	B. Braun
MolYsis-SelectNA™plus	Molzym
SelectNA™*plus*	Molzym
S-Monovette®	Sarstedt

Technical Support

If you have questions please call us.

Our hotline: +49(0)421 69 61 62 0 • **E-Mail:** support@molzym.com

Material safety data sheets are available on request.

Also visit Molzym's homepages for further information:
http://www.molzym.com

Technical Service

The maintenances of the instrument should be done on a yearly basis. For further information consult the technical service.

Contact

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Order Information

Product	Contents and Application	Cat. No.
MolYsis-SelectNA™plus (research use only)	**48** reactions Automated pathogen DNA isolation	D-450-048
SelectNA™*plus* necessary for processing of the *MolYsis-SelectNA™plus*.	**Instrument** for automated pathogen DNA extraction for 1 to 12 samples of whole blood, other body fluids, swabs and tissues.	D-400-001

Other Products supplementary to *MolYsis-SelectNA™plus*

Product (research use only)	Contents and Application	Cat. No.
Pipette tips* SelectNA™*plus, DNA-free Necessary for processing of the *MolYsis-SelectNA™plus*.	**2x [2x 96]** tips **4x [2x 96]** tips **8x [2x 96]** tips	D-925-024 D-925-048 D-925-096
Waste bags* SelectNA™*plus Necessary for processing of the *MolYsis-SelectNA™plus*.	**500** bags	D-928-500
Cleaning Cartridges for the SelectNA™*plus* instrument.	**12** cartridges	D-927-012
UMD Tubes Sample storage	**20** vials: Storage of blood and other primary body fluids. Sample volume: 0.4 to 2 ml	Z-801-020
Mastermixes and PCR Reagents, DNA-free (2.5x concentrated)		
Mastermix 16S Complete Universal 16S rDNA PCR and Real-Time PCR assay for detection of bacterial DNA.	**100** reactions **250** reactions **1000** reactions	S-020-0100 S-020-0250 S-020-1000
Mastermix 18S Complete Universal 18S rDNA PCR and Real-Time PCR assay for detection of fungal DNA.	**100** reactions **250** reactions **1000** reactions	S-070-0100 S-070-0250 S-070-1000
MolTaq 16S/18S Taq DNA Polymerase, DNA-free	**100** units **500** units	P-019-0100 P-019-0500
DNA-free water, *PCR grade*	**10x** 1.7 ml	P-020-0003

Order Hotline:

Tel.: +49(0)421 69 61 62 0 • **Fax:** +49(0)421 69 61 62 11 • **E-Mail:** order@molzym.com