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Safety Information for Sample Pre-Treatment and DNA Isolation

Component	Classification and Hazard / Precautionary Statements* (page 2)
Buffer *PKB* **Control buffer,** prefilled in *Buffer cartridges* (W5; page 6)	Contains sodium dodecyl sulfate (< 10 %): **Acute toxicity (oral, inhalation), irritation (skin and eye)** H302-H315-H319-H332; P280-P301+P312-P304+P340+P312-P305+P351+P338  **Warning**
Proteinase K Enzyme K	Contains *Proteinase K* (≥ 1 %) **Respiratory sensitization and skin sensitization** H317-H334; P280-P302+P352-P333+P313-P363  **Danger**
BugLysis plus	Contains 2-mercaptoethanol (< 10 %) **Acute toxicity (skin), eye damage, skin sensitization, reproductive toxicity and hazardous to aquatic environment (chronic)** H310-H317-H318-H361d-H411; P273-P280-P301+P310-P302+P352+P310-P305+P351+P338  **Danger**
Lysis buffer, prefilled in *Buffer cartridges* (W0; page 6)	Contains guanidine hydrochloride (> 10 %) **Acute toxicity (oral) and irritating (eyes and skin)** H302-H315-H319; P301+P312-P302+P352-P305+P351+P338  **Warning**
Binding buffer, prefilled in *Buffer cartridges* (W6; page 6)	Contain 2-propanol (< 40 %) and 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).** H225-H302-H312-H314-H319-H336-H412-EUH032; P210-P233-P280-P303+P361+P353-P305+P351+P338-P310-P362+P364  **Danger**

***Please note:** **Before using this scheme inform yourself of the details of the procedure.** Special care is required for working under DNA-free conditions and secure working conditions. **Please consult the manual for more information.**

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Safety Information for Sample Pre-Treatment and DNA Isolation

Component	Classification and Hazard / Precautionary Statements*
Washing buffer, prefilled in *Buffer cartridges* (W7; page 6)	Contains ethanol (> 50 %) **Flammable liquids and irritating (eyes)** H225-H319; P210-P233-P305+P351+P338

Danger

Important notes: When working with chemicals, always wear suitable protective lab clothing and work in a Class II biological safety cabinet.

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

The *lysis buffer* (W0) and *binding buffer* (W6) prefilled in *Buffer cartridge* contain guanidine salts, which can form highly reactive compounds and toxic gases when combined with hypochlorite or other acidic solutions.

For more information, please consult the appropriate material safety data sheets (MSDS) which are available on request.

Emergency call: 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.

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Micro-Dx / Sample Preparation*

Kit 1 DNA Isolation Unit: Buffers and Consumables
Kit 2 DNA Isolation Unit: Enzymes (-15 to -25°C) for enzymatic pre-treatment

- Work under a laminar flow, Class II biological safety cabinet.
- All specimens should be sampled under aseptic conditions.
- Transport the sample under conditions avoiding contamination to the laboratory for analysis.
- For each sample place a *Plus-SV* vial (screw cap, Kit 1) and an *ET* tube (Kit 1) in a rack, close lid and mark with sample ID. Do not mark *Plus-SV* vials on the lid.

Fluid Sample Preparation

1. Transfer of the fluid specimens:
   a) **Method 1:** Clear or cloudy fluid samples (without enzymatic pre-treatment). Pipette up to 1 ml of the fluid specimen into a *Plus-SV* vial.

   b) **Method 2**: Mucous fluids, purulent fluids and fluids with flakes of tissue or solid particles (with enzymatic pre-treatment).
   - Place a ***ST*** tube (flip cap, Kit 1) in a rack and mark with sample ID.
   - Pipette up to 0.8 ml fresh fluid sample into a *ST* tube. Less sample volume fill up to 0.8 ml with buffer *SU*. For difficult sample material, use less material (e.g., 0.3 to 0.5 ml) and fill up to 0.8 ml with buffer *SU*.
   - Add **180 µl of buffer *PKB*** (Kit 1) and **20 µl of *Enzyme K*** (Kit 2D).
   - **Vortex** for 15 s and **incubate** at 56°C, 10 min, 1,000 rpm in a thermomixer.
   - Transfer the fluid phase into a ***Plus-SV*** vial (screw cap) by pipetting.

⚠ **Avoid transferring any particles!**

2. Check that 1 ml sample volume is contained in the *Plus-SV* vial, fill up to 1 ml with buffer *SU*, if required (use the measure line of the tube).
3. Then mix by pipetting.
4. Transport the rack with closed *Plus-SV* vials and *ET* tubes to the instrument.

⚠ **Do not use the *ST* tubes (flip cap) in the instrument. Only for pre-treatment!**

Fluid

Continue with the instructions of the scheme Micro-Dx / Automated DNA Isolation (page 5).

Swab Sample Preparation

1. Place a ***ST*** tube (flip cap, Kit 1) in a rack and mark with the sample ID.
2. Pipette 1 ml of buffer *SU* (Kit 1, package E) into the *ST* tube. Remove the swab from the swab vial and transfer to the *ST* tube.

   Note: If available, pipette 1 ml fluid from the swab vial into a *ST* tube instead of buffer *SU*. In case there is less than 1 ml fluid available, fill up to 1 ml with buffer *SU* (use the measure line of the tube).
3. Wash the swab by swirling it in the fluid of the *ST* tube and pressing it to the tube wall several times. Discard the swab. (Continue step 4, page 4)

Swab

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Swab

4. Transfer the sample from the *ST* tube into a *Plus-SV* vial (screw cap).
5. Check that 1 ml sample volume is contained in the *Plus-SV* vial, fill up to 1 ml with buffer *SU*, if required (use the measure line of the tube).
6. Then mix by pipetting.
7. Transport the rack with closed *Plus-SV* vials and *ET* tubes to the instrument.

Do not use the *ST* tubes (flip cap) in the instrument. Only for pre-treatment!

Continue with the instructions of the scheme Micro-Dx / Automated DNA Isolation (page 5).

Tissue Sample Preparation

Tissue

1. Place a ***ST* tube** (flip cap; Kit 1) in a rack and mark with the sample ID.
2. Pipette **180 µl** of buffer ***PKB*** (Kit 1) into the *ST* tube.
3. Transfer the sample to a sterile support (e.g., Petri dish) by using sterile forceps.
4. Cut the sample (~0.5 x 0.5 x 0.5 cm) into small pieces by using a sterile scalpel.
5. Transfer the dissected specimen to the *ST* tube filled with buffer *PKB*. The specimen should be covered completely by the buffer.
6. Add **20 µl** of ***Enzyme K*** (Kit 2) into the buffer in the *ST* tube.
7. Vortex for **15 s** and **incubate** at 56°C, 10 min, 1,000 rpm in a thermomixer.
8. Transfer the fluid phase into a *Plus-SV* vial (screw cap) by pipetting (use 200 µl pipette).

Optional, in case of difficult sample transfer, fill up to 1 ml with buffer *TSB*. **Vortex** for **15 s** and **centrifuge** for **5 s** (up to 2,000 xg). Transfer fluid phase into *Plus-SV* vial by pipetting (200 µl pipette). Continue with step 9.

Avoid transferring any particles!

9. Check that 1 ml sample volume is contained in the *Plus-SV* vial, fill up to 1 ml with the transport solution, if available, or with buffer TSB (Kit 1). For this, use the measure line of the tube. **Avoid transferring any particles from the transport solution!**
10. Mix the sample by pipetting.
11. Transport the rack with closed *Plus-SV* vials and *ET* tubes to the instrument.

Do not use the *ST* tubes (flip cap) in the instrument. Only for pre-treatment!

Continue with the instructions of the scheme Micro-Dx / Automated DNA Isolation (page 5).

Please note:** **Before using this scheme inform yourself of the details of the procedure.** Special care is required for working under DNA-free conditions and secure working conditions. **Please consult the manual for more information.

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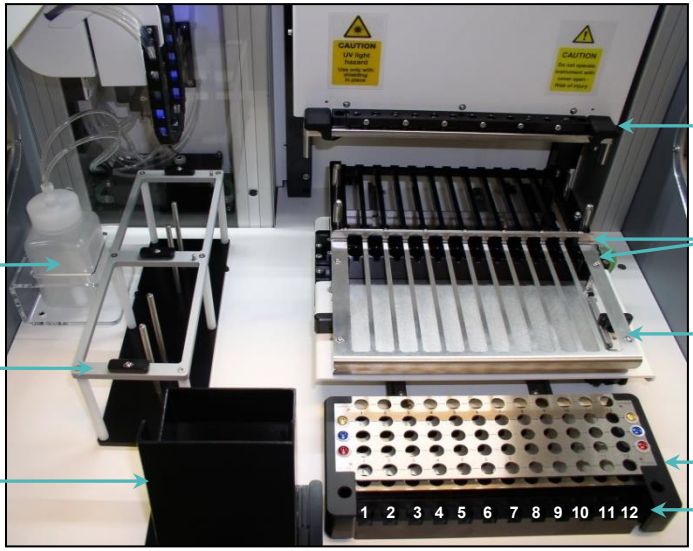
Kit 1 DNA Isolation Unit: Buffers and Consumables (+18 to +25°C)
Kit 2 DNA Isolation Unit: Enzymes (-15 to -25°C)



Positions:

Holder for *Extraction columns*

Extraction cartridge rack

Heating block with cooling tubes

Buffer cartridge rack

Reagent vial rack with safety cover, if available

Position numbers

Reservoir with pipetting solution

Pipette tip holder

Waste chute

Decontamination of the instrument

- Ensure that the instrument is UV-decontaminated before each extraction run (main menu: 'UV decontamination').

Before starting

- Briefly centrifuge the enzyme vials (Kit 2; one vial each of *MolDNase C*, *BugLysis plus* and *Proteinase K* for each sample) to clear the lid.
- Store vials in a rack (-15 to -25°C) for further usage (page 6).

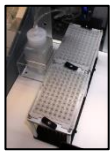
Loading the instrument

1. Load the *Waste bag* to the waste chute.

2. Check that the reservoir is filled with enough pipetting solution (250 ml autoclaved deionized water, fill level mark blue arrow).

3. Select script 1 'SelectNAplus' and the number of samples. Load the following components and confirm every loading step by pressing the control button.

4. Load *Pipette tips*.
   Be sure that there are enough filled tip rows to run the selected number of samples.



Please note:** **Before using this scheme inform yourself of the details of the procedure.** Special care is required for working under DNA-free conditions and secure working conditions. **Please consult the manual for more information.

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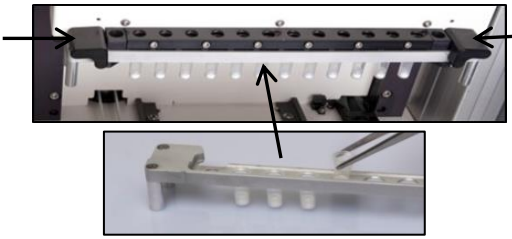
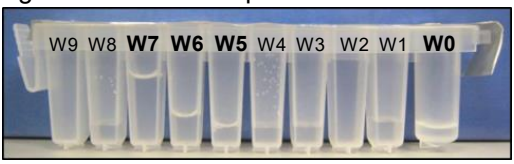
Please note: All racks must be loaded from the left to the right side (position 1 to 12).

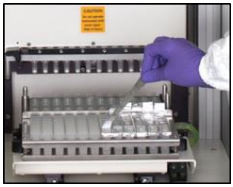
5. Load *Extraction columns* and lock by the two clips.



6. Load *Extraction cartridges* to the rack, place in the instrument and lock by the two clips.

7. Load *Buffer cartridges* to the rack and place it in the instrument and lock by the two clips. Peel off the aluminium foil by pulling constantly slightly to the right hand side from position 1 to 12.





8. Close the door and press button.

⚠ The cartridge rack moves backwards. Keep your hands of the instrument!

Open the door. Load the reagent vial rack. Place the following vials in sequential order in the rack from left to right (positions 1 to 12). Remove caps of the vials in following order:

1. *BugLysis plus* (yellow capped)
2. *Proteinase K* (blue capped)
3. *MolDNase C* (red capped)
4. Elution tubes (*ET*, flip cap)
5. Plus-Sample vials (*Plus-SV*, screw cap)

Place the safety cover on the reagent vial rack, if available.

Starting the instrument

9. Check that the aluminium foil of the *Buffer cartridges* and all caps of the vials are removed.
10. Close the door and press the control button to start the extraction.

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Symbols and explanation of the PCR working places:

DNA-free Work under a PCR UV workstation. Use components of **Kit 3.** For the preparation of mastermixes *MA Bac*, *MA Yeasts* and *MA Control*.

DNA Work under a UV laminar flow hood (Class II), where samples are prepared. Use components of **Kit 4B.**
For the preparation of:
- Sample loading into the mastermixes
- Handling of positive PCR controls *P1* and *P2*

Places Handlings Performed

DNA-free

Assay Bacteria (*MA Bac*)
- 1 reaction per sample
- 2 reactions for the positive controls (P1, P2)
- 1 reaction for negative control (NC Bac)

Assay Yeasts (*MA Yeasts*)
- 1 reaction per sample
- 2 reactions for the positive controls (P1, P2)
- 1 reaction for negative control (NC Yeasts)

Assay Control (*MA Control*)
- 1 reaction per sample
- 1 reaction for negative control (NC IEC)

PCR Assaying

Before starting the mastermix preparation:

DNA-free

- **Kit 3:** Thaw the following vials of at room temperature (+18 to +25°C):
  - H_2O
  - *MA Bac* (2.5x conc.)
  - *MA Yeasts* (2.5x conc.)
  - *MA Control* (2.5x conc.)
  - *DS* (light sensitive)

  Vortex thawed PCR reagent vials for a few seconds to mix and briefly centrifuge to clear the lid.

DNA

- **Kit 4B:** Thaw the following vials at room temperature (+18 to +25°C):
  - *DNA Standard P1*
  - *DNA dilution buffer* (for P1)

  Preparation of Positive PCR Control P2

  1. Vortex *P1* and *DNA dilution buffer* vials and pulse centrifuge.
  2. Pipette **998 µl** *DNA dilution buffer* in a 1.5 ml tube (DNA- and DNase-free; not supplied)
  3. Add **2 µl** *P1* and vortex to mix.
  4. Briefly centrifuge to clear lid.
  5. Label dilution with *‚P2‘* and the preparation date.

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Keep all PCR tubes filled with **mastermixes** and the ***MolTaq 16S/18S*** chilled in the cooling racks **(-15 to -25°C)**. Do not interrupt the cooling. ***Cooling*** of the PCR tubes ***is important*** to minimize the generation of primer dimers.

DNA-free

1. **Arrange PCR tubes** (not supplied) in a cooling rack and mark.
2. **Briefly** centrifuge *MolTaq 16S/18S* and place it in the cooling rack (-15 to -25°C).
3. **Place a *MT* tube** for each mastermix in a cooling rack. **Pipette the following components** into each tube (see 'PCR Assaying' page 1, short manual). Preparation of mastermix see Table 1. Vortex tube to mix and briefly centrifuge.

Table 1: Preparation of mastermixes (Kit 3). Component volumes in µl.

reactions	*MA Bac, MA Yeasts or MA Control*	*H_2O*	*DS*	*MolTaq 16S/18S*
1	10.0	7.5	2.5	0.8
2	20.0	15.0	5.0	1.6
3	30.0	22.5	7.5	2.4
4	40.0	30.0	10.0	3.2
5	50.0	37.5	12.5	4.0
6	60.0	45.0	15.0	4.8
7	70.0	52.5	17.5	5.6
8	80.0	60.0	20.0	6.4
9	90.0	67.5	22.5	7.2
10	100.0	75.0	25.0	8.0

4. **Pipette 20 µl** of each mastermix into the chilled (-15 to -25°C) PCR tubes per reaction (dedicated for samples, PC and NC, respectively).
5. **Add 5 µl** *H_2O* as PCR negative control (NC). Close PCR tubes.
6. **Place PCR tubes** in another cooling rack for template loading.

DNA

7. **Add 5 µl** of each sample eluate into the mastermixes.
8. **Add 5 µl** PCR positive controls P1 and P2, respectively.
9. **Start PCR program of Eppendorf Mastercycler (manual section 'PCR Detection", part "2C) PCR Thermocycling')**. See chapter "Addendum" of the manual for other cyclers.

Preparation of Mastermixes

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Important notes before starting

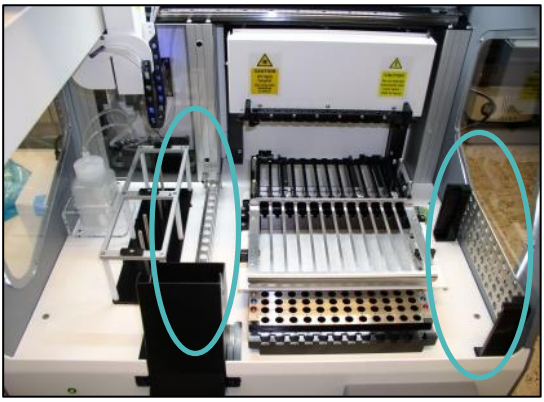
To be supplied by the user

- low-lint soft paper towel
- Disinfectant e.g., Meliseptol® (Braun, Germany) or 70 % (v/v) ethanol
- Bleach solution (1 % active Cl_2)

Do not spray surface inside the instrument with disinfectant or other fluids. Instead use a paper towel soaked with disinfectant to wipe surfaces.

Discard *waste bag* including used components form the SelectNA*plus* instrument as infectious waste according to your institution's procedures.

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 %).



The interior of the cleaned instrument.

General Information

Yearly maintenace

- The maintenance of the instrument should be done on a yearly basis.
- Please contact your service engineer to run the yearly maintenance on your instrument.

Yearly

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Decontamination after each run

After Each Run

- Dispose the empty *Plus-SV* vials and enzyme vials.
- Spray the waste chute with disinfectant, let it incubate for 10 min.
- Remove the Reagent vial rack, wipe the surface with disinfectant and place it back.
- Press button to transfer rack (see control board).
- Remove waste bag.
- Dispose *Buffer cartridges*, *Extraction cartridges* and *Extraction columns* in the Waste bag, **but not** via the waste chute.
- Wipe the removed racks, suction cups and surfaces around the instrument with disinfectant, wait specified time of exposure and place the dry racks back.
- Place the column rack to the right side of the pipette tip holder for UV cleaning (see Fig. page 7, left circled marking).
- Place the Safety cover of the Reagent vial rack to the right side of the instrument for UV cleaning (see Fig. page 7, right circled marking).
- Check the fill level of the reservoir (use bottle holder as a marker for minimum fill level of pipetting solution).
- Remove Waste chute after 10 min incubation and wipe with soft paper towel (soaked with disinfectant). If necessary, clean the waste chute in a laboratory dishwasher.
- Close the door, clean the door handle and the door top.
- Select and start 'UV decontamination' program from the 'Main Menu'.

Every 14 days decontamination of the instrument

14-day

- Follow the "Decontamination after each run" instructions.
- Select 'Cleaning script' from the 'Main Menu'.
- Following the instructions given on instrument display to clean tubing system with 1 % bleach and autoclaved deionized water.
- Cleaning of the pipetting system by using the *Cleaning bottle*, 4 *Cleaning cartridges*, 1 % bleach solution and autoclaved deionized water.
- Clean both bottles (*Cleaning bottle* and reservoir for pipetting solution) by shaking with 1 % bleach. Use the screw cap of the *Cleaning bottle* for this procedure.
- Clean lid (incl. tubing for the pipette system) with a low-lint, soft paper towel soaked with the 1 % bleach solution.
- Clean the white suction cups of the vacuum system with a paper towel soaked with disinfectant.

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