molzym
reveal DNA

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**

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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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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.
- Transport the sample under conditions avoiding contamination to the laboratory for analysis.

Fluid Sample Preparation

Fluid

- Transfer of the fluid specimens:
  Method 1: Clear or cloudy fluid samples (without enzymatic pre-treatment).
  Place a *Plus-SV* (screw cap vial; Kit 1) in a rack and mark with the sample ID. Do not mark vials on the lid.
  Pipette 1 ml of the fluid specimen into a *Plus-SV* vial. In case of less sample volume than 1 ml, fill up to 1 ml with buffer SU (Kit1) (use the measure line of the tube).

  Method 2: Mucous fluids, purulent fluids and fluids with flakes of tissue or solid particles (with enzymatic pre-treatment).
  Pipette 0.8 ml fresh fluid sample into a ***ST* tube**. Add 180 µl of buffer *PKB* (Kit 1) and **20 µl of *Enzyme K*** (Kit 2D), **vortex** for 15 s. **Incubate** at 56°C, 10 min, 1,000 rpm in a thermomixer.
  Transfer the fluid phase into a ***Plus-SV*** vial (screw cap vials, Kit 1) by pipetting. **Avoid transferring any particles.** Mark the vial with the sample ID (not on the lid).
- Transport the rack with closed *Plus-SV* vials and *ET* tubes (Kit 1) to the instrument.

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

Swab Sample Preparation

Swab

- Place a *ST* tube (flip cap tube for swabs & enzymatic pre-treatment; Kit 1) in a rack and mark with the sample ID.
- Pipette 1 ml of buffer *SU* (Kit 1, package E) into 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 by pipetting buffer *SU* (use the measure line of the tube).
- 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.
- Transfer the sample from the *ST* tube into a *Plus-SV* vial (screw cap vial) and mark the vial with sample ID. Do not mark on the lid.
- Transport the rack with closed *Plus-SV* vials and *ET* tubes (Kit 1) to the instrument.

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

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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.
- Transport the sample under conditions avoiding contamination to the laboratory for analysis.

Tissue Samples

- Per specimen, place a *ST* tube (flip cap tube for swabs & enzymatic pre-treatment; Kit 1) in a rack and mark with the sample ID. Pipette 180 µl of buffer *PKB* (Kit 1) into the *ST* tube.
- Transfer the sample to a sterile support (e.g., Petri dish). Cut the sample (~0.5x0.5x0.5 cm) into small pieces by using a sterile scalpel.
- 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 2), **vortex for 15 s.**
  Incubate at 56°C, 10 min, 1,000 rpm in a thermomixer.
- Transfer the fluid phase into a *Plus-SV* vial (screw cap vial; mark with sample ID; do not mark on the lid) by pipetting. **Avoid transferring any particles.**
- 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.
- Transport the rack with closed *Plus-SV* vials and *ET* tubes (Kit 1) to the instrument.

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

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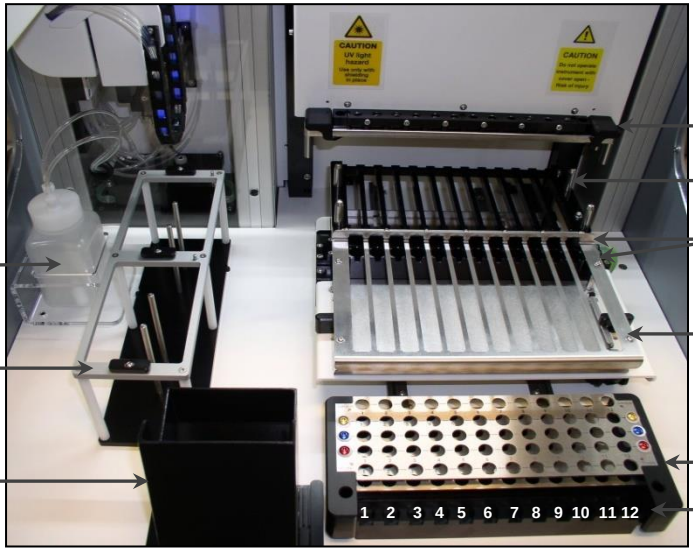
Kit 1 DNA Isolation Unit: Buffers and Consumables (+18 to +25°C)
Kit 2 DNA Isolation Unit: Enzymes (-15 to -25°C)



Decontamination of the instrument

- The instrument must be **UV decontaminated** (Main Menu: **'**UV decontamination') before an extraction run is started.

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).

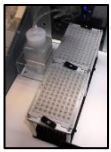
Loading the instrument

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

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).

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



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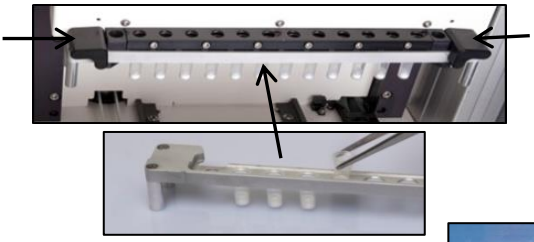
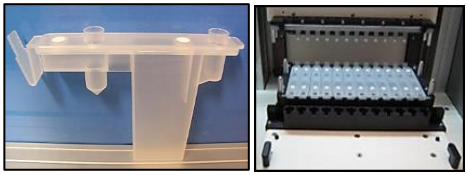
Please note: All racks must be loaded from the left to the right side (positon 1 to 12).

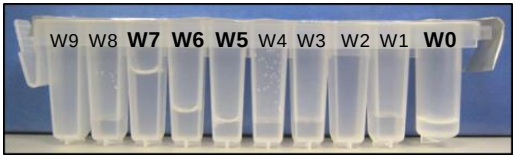
4. Load *Extraction columns* and lock by the two clips.

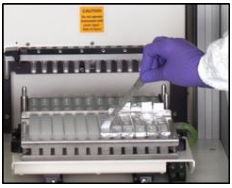


5. Load *Extraction cartridges* to the rack, place in the instrument and lock by the two clips.



6. Load *Buffer cartridges* to the rack and place it in the instrument. Peel off the aluminium foil by pulling constantly slightly to the right hand side from positon 1 to 12.





7. Close the door. Caution: The cartridge rack moves backwards. Keep your hands off the instrument!

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

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*)

Place the safety cover on the reagent vial rack.

Starting the instrument

- Check that the aluminium foil of the *Buffer cartridges* and all caps of the vials have been removed. 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 assays
- Handling of positive PCR controls *P1* and *P2*;
  Preparation of *P2* (998 µl DNA dilution buffer + 2 µl *P1*)

Places where Handlings are performed

DNA-free

Thaw the following vials at room temperature (+18 to +25°C):

Kit 3:

- H_2O
- 2.5x *MA Bac*
- 2.5x *MA Yeasts*
- 2.5x *MA Control*
- *DS;* keep dark

DNA

Kit 4B:

- *DNA Standard P1*
- *DNA dilution buffer* (for P1)

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

DNA-free

MA Bac

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

MA Yeasts

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

MA Control

- 1 reaction per sample
- 1 reaction for negative control (NC IEC)

PCR Assaying

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Note: Keep all PCR tubes filled with **PCR-ready 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

DNA

- **Pipette the following components of the mastermix assays** (*MA Bac, MA Yeasts* and *MA Control*, Kit 3) **into each *MT* tube** (Kit 1). Preparation see **Tab. 1**.

Tab. 1: Preparation of PCR-ready mastermixes (Kit 3). 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

- Vortex PCR-ready mastermixes and centrifuge briefly.
- Pipette into the chilled (-15 to -25°C) PCR tubes per reaction:

  20 µl of PCR-ready mastermix ***MA Bac, MA Yeasts*** or ***MA Control***.

  Add **5 µl** *H_2O* as negative control.

  Add **5 µl** sample eluate and, *P1* and *P2*, respectively.

- **Start the PCR programme of Eppendorf Mastercycler** (manual chapter “PCR Detection”, part “2C) PCR Thermocycling”)

  See chapter “Addendum”, part “Real-Time PCR Thermocycling and Detection by Melting Curve Analysis” of the manual for other cyclers.

Preparation of Mastermixes
MA Bac, MA Yeasts or MA Control

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General Information

Important notes before starting

To supply 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).

Note

- Do not spray surface inside the instrument with disinfectant or other fluids.

The interior of the cleaned instrument.

After each run

Decontamination after each run

- Dispose the empty *Plus-SV* vials and enzyme vials.
- Spray the waste chute with disinfectant, wait for 10 min and wipe with soft paper towel.
- Remove the Reagent vial rack and wipe the surface with disinfectant.
- Press button to transfer rack (see control board).
- Remove waste bag (if necessary).
- 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 and place the racks back.
- Place the column rack to the right side of the pipette tip holder for UV cleaning (see Fig. above).
- Place the Safety cover of the Reagent vial rack to the right side of the instrument for UV cleaning.(see Fig. above).
- Close the door, wipe the door handle and the door top.
- Select UV decontamination program.

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Daily decontamination of the instrument

Daily

- Follow the cleaning instruction after each run.
- Remove the waste chute and clean it. Spray the waste chute with the disinfectant, wait for 10 min.
- After incubation wipe the waste chute with soft paper towel. If necessary clean the waste chute in a washing machine.
- Place it back to the instrument.
- Close the door and select UV decontamination

Every 14 days decontamination of the instrument

14-day

- Follow the “Decontamination after each run” and “Daily decontamination of the instrument” instructions.
- Select Cleaning script.
- 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* and 1 % bleach solution.
- 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 both lids (with tubing for the pipette system) with a low-lint, soft paper towel soaked with the 1 % bleach solution.

Yearly maintenace

Yearly

- 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.

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