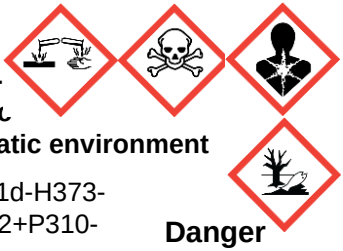
molzym reveal DNA

Safety Information for Sample Pre-Treatment and DNA Isolation

Component	Classification and Hazard / Precautionary Statements*(page 2)
Buffer *CM*	Contains guanidine hydrochloride (> 10 %) **Acute toxicity (oral) and irritating (eyes and skin)** H302-H315-H319; P301+P312-P302+P352-P305+P351+P338  **Warning**
ß-mercapto-ethanol	Contains 2-mercaptoethanol (100%, CAS no. 60-24-2): **Acute toxicity, irritation (skin), eye damage, skin sensitization, reproducetive toxicity, specific target organ toxicity, hazardous to aquatic environment** H301+H331-H310-H315-H317-H318-H361d-H373-H410; P273-P280-P301+P310-P302+P352+P310-P304+P340+P310-P305+P351+P338  **Danger**
Proteinase K	Contains *Proteinase K* (≥ 1 %): **Respiratory sensitization and skin sensitization** H317-H334; P280-P302+P352-P333+P313-P363  **Danger**
Buffer *RP* **Buffer *PKB***	Contains sodium dodecyl sulfate (< 10 %): **Acute toxicity (oral, inhalation), irritation (skin and eye)** H302-H315-H319-H332; P280-P301+312-P304+340+312-P305+351+338  **Warning**
Buffer CS	Contains guanidinium thiocyanate (> 10 %): **Acute toxicity (oral), skin sensitization, eye damage and hazardous to aquatic environment (chronic)** H302-H312-H314-H318-H412-EUH032; P280-P303+P361+P353-P305+P351+P338-P310-P362+P364  **Danger**
Buffer *AB* **Buffer *WB***	Contains 2-propanol (AB > 40 % and WB ≥ 40 %): **Flammable liquids and irritating (eyes)** H225-H319-H336; P210-P233-P305+P351+P338  **Danger**

***Please note:** **Before using this scheme inform yourself of the details of the procedure. Please consult the manual.** 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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Version 05

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Safety Information for Sample Pre-Treatment and DNA Isolation

Component	Classification and Hazard / Precautionary Statements*
70% Ethanol	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.

Buffers *CM* and *CS* 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 fertility. Suspected of damaging the unborn child; **H373:** May cause damage to organs (liver, heart) through prolonged or repeated exposure if swallowed; **H301+H331:** Toxic if swallowed or if inhaled.**H410:** Very 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; **P363**: Wash contaminated clothing before reuse; **P301+P310:** IF SWALLOWED: Immediately call a POISON CENTER or doctor; **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+P310:** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor; **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; **P362+P364:** Take off contaminated clothing and wash it before reuse.

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Protocol 1, part A: Fluid Sample Preparation

Kit 1: Buffers & Consumables (+18 to +25°C)

- Place a *Sample tube* (*ST*; Kit 1) in a rack and mark.
- Pipette 1 ml of the fluid specimen in the *Sample tube* (*ST*).
- In case of less sample volume available, pipette the fluid into the *Sample tube* (*ST*) and fill up to 1 ml with buffer *SU* (Kit 1) (use the measure line of the tube).

Continue with the instructions of the scheme Ultra-Deep Microbiome Prep / Protocol 1, part B: DNA Isolation (page 5, short manual).

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Protocol 2, part A: Tissue Sample Preparation

Kit 1: Buffers & Consumables (+18 to +25°C)
Kit 2: Enzymes & Reagents (-15 to -25°C)

- Transport the specimen under conditions avoiding contamination to the laboratory for analysis.
- Per specimen, place a *Sample tube* (*ST*, Kit 1,) in a rack and mark. Pipette 180 µl of buffer *PKB* (Kit 1) into the *Sample tube* (*ST*).
- Transfer the specimen to a sterile support (e.g., Petri dish). Cut the specimen (~0.5 x 0.5 x 0.5 cm) into small pieces by using a sterile scalpel.
- Transfer the cut specimen to the *Sample tube* (*ST*) filled with buffer *PKB.* **Add 20 µl of *Proteinase K*** (Kit 2), **vortex for 15 s.** **Incubate** at 56°C, 10 min, 1,000 rpm (thermomixer).
- Fill up to 1 ml with the transport solution, if available, or with buffer *TSB* (use the measure line of the tube).

Continue with the instructions of the scheme Ultra-Deep Microbiome Prep / Protocol 2, part B: DNA Isolation (page 7, short manual).

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Ultra-Deep Microbiome Prep
Protocol 1, part B: DNA Isolation*

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Kit 1: Buffers & Consumables (+18 to +25°C)
Kit 2: Enzymes & Reagents (-15 to -25°C)

Arrange bottles according to the sequence of steps as below:
CM – DB1 – RS – RL – RP – CS – AB – WB –70% Ethanol – Deionized water

Continued from Ultra-Deep Microbiome Prep / Fluid Sample Preparation, part A (page 3, short manual).

Per sample:

Depletion of Human DNA

1. Add **250 µl buffer *CM***, vortex for 15 s.
   Let stand at room temperature (+18 to +25°C) for 5 min.
2. Briefly centrifuge.
   Add **250 µl buffer *DB1***.
   Add **10 µl *MolDNase B*** (Kit 2), vortex for 15 s.
   Incubate at room temperature (+18 to +25°C) for 15 min.
3. Centrifuge at ≥12,000x*g*, 10 min.
   Remove supernatant by pipetting and discard.
4. Resuspend pellet in **1 ml buffer *RS*** by pipetting.
5. Centrifuge at ≥12,000x*g*, 5 min.
   Remove supernatant by pipetting.
   (Optional: freeze pellet at -15 to -25°C for storage).

Lysis of Pathogens

6. Resuspend pellet in **80 µl buffer *RL***, briefly centrifuge tube.
7. Add **20 µl *BugLysis*** (Kit 2).
   Add **1.4 µl *ß-mercaptoethanol*** (Kit 2), vortex for 15 s.
   Take care not to inhale.
   Incubate at 37°C, 30 min, 1,000 rpm (thermomixer).
8. Briefly centrifuge.
   Add **150 µl buffer *RP***.
   Add **20 µl *Proteinase K*** (Kit 2), vortex for 15s.
   Incubate at 56°C, 10 min, 1,000 rpm (thermomixer).

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***Please note:** **Before using this scheme inform yourself of the details of the procedure. Please consult the manual.** 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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During 10 min incubation:

Kit 1: Buffers & Consumables

Unpack *Spin columns* (*SC*), 2 ml *Collection tubes* (*CT*) and 1.5 ml *Elution tubes* (*ET*), label; heat ***Deionized water*** (100 µl each sample) vial to **70°C** (thermomixer).

Lysis of Pathogens

9. Briefly centrifuge.
   Add **250 µl buffer *CS***, vortex for 15 s.

10. Briefly centrifuge.
    Add **250 µl buffer *AB***, vortex for 15 s.

DNA Purification

11. Briefly centrifuge to clear lid.
    Pipette lysate into a *Spin column.*
    Centrifuge: ≥12,000x*g*, 30 to 60 s.

12. Remove column and place in a new 2 ml *Collection tube.*
    Add **400 µl buffer *WB***.
    Centrifuge: ≥12,000x*g*, 30 to 60 s.

13. Remove column and place in a new 2 ml *Collection tube.*
    Add **400 µl *70% Ethanol***.
    Centrifuge: ≥12,000x*g*, 3 min.

DNA Elution

14. Carefully remove column and place in a 1.5 ml *Elution tube*.

15. Add **100 µl *Deionized water*** heated to 70°C.
    Incubate at room temperature (+18 to +25°C) for 1 min.
    Centrifuge: ≥12,000x*g*, 1 min.
    Discard column, close lid of *Elution tube*.

16. Store eluted DNA (1.5 ml *Elution tube*) at -15 to -25°C.

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Kit 1: Buffers & Consumables (+18 to +25°C)
Kit 2: Enzymes & Reagents (-15 to -25°C)

Arrange bottles according to the sequence of steps as below:
CM – DB1 – RS – RL – RP – CS – AB – WB –70% Ethanol – Deionized water

Continued from Ultra-Deep Microbiome Prep / Tissue Sample Preparation, part A (page 4, short manual).

Per sample:

Depletion of Human DNA

1. Add **250 µl buffer *CM***, vortex for 15 s.
   Let stand at room temperature (+18 to +25°C) for 5 min.
2. Briefly centrifuge.
   Add **250 µl buffer *DB1***.
   Add **10 µl *MolDNase B*** (Kit 2), vortex for 15 s.
   Incubate at room temperature (+18 to +25°C) for 15 min.
3. Centrifuge at ≥12,000x*g*, 10 min.
   Remove supernatant by pipetting and discard.
4. Resuspend pellet in **1 ml buffer *RS*** by pipetting.
5. Centrifuge at ≥12,000x*g*, 5 min.
   Remove supernatant by pipetting.
   (Optional: freeze pellet at -15 to -25°C for storage).

Lysis of Pathogens

6. Resuspend pellet in **80 µl buffer *RL***, briefly centrifuge tube.
   Add **20 µl *BugLysis*** (Kit 2).
   Add **1.4 µl *ß-mercaptoethanol*** (Kit 2), vortex for 15 s.
   Take care not to inhale.
   Incubate at 37°C, 30 min, 1,000 rpm (thermomixer).
7. Briefly centrifuge.
   Add **150 µl buffer *RP***.
   Add **20 µl *Proteinase K*** (Kit 2), vortex for 15 s.
   Incubate at 56°C, 10 min, 1,000 rpm (thermomixer).

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During 10 min incubation:

Kit 1: Buffers & Consumables

Unpack *Spin columns* (*SC*), 2 ml *Collection tubes* (*CT*) and 1.5 ml *Elution tubes* (*ET*), label; heat ***Deionized water*** (100 µl each sample) vial to **70°C** (thermomixer).

Lysis of Pathogens

8. Briefly centrifuge.
   Add **250 µl buffer *CS***, vortex for 15 s.

9. Briefly centrifuge.
   Add **250 µl buffer *AB***, vortex for 15 s.

DNA Purification

10. Briefly centrifuge to clear lid.
    Pipette lysate into a *Spin column.*
    Pipette the fluid phase in the column.
    Avoid transfer of any unresolved particles!
    Centrifuge: ≥12,000x*g*, 30 to 60 s.

11. Remove column and place in a new 2 ml *Collection tube.*
    Add **400 µl buffer *WB***.
    Centrifuge: ≥12,000x*g*, 30 to 60 s.

12. Remove column and place in a new 2 ml *Collection tube.*
    Add **400 µl *70% Ethanol***.
    Centrifuge: ≥12,000x*g*, 3 min.

DNA Elution

13. Carefully remove column and place in a 1.5 ml *Elution tube*.

14. Add **100 µl *Deionized water*** heated to 70°C.
    Incubate at room temperature (+18 to +25°C) for 1 min .
    Centrifuge: ≥12,000x*g*, 1 min.
    Discard column, close lid of *Elution tube*.

15. Store eluted DNA (1.5 ml *Elution tube*) at -15 to -25°C.

***Please note:** **Before using this scheme inform yourself of the details of the procedure. Please consult the manual.** 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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