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 1ml 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 1ml 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 3, short manual).

***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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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 180µl of buffer *PKB* (Kit 1) into the *Sample tube* (*ST*).
- Transfer the specimen to a sterile support (e.g., Petri dish). Cut the specimen (~0.5x0.5cm) into small pieces by using a sterile scalpel.
- Transfer the cut specimen to the *Sample tube* (*ST*) filled with buffer *PKB.*
  Add 20µl of *Proteinase K* (Kit 2), **vortex for 15s.**
  Incubate at 56°C, 10min, 1,000rpm (thermomixer).
- Fill up to 1ml 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 5, short manual).

***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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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 1, short manual).

Per sample:

1. Add **250µl buffer *CM***, vortex for 15s.
   Let stand at room temperature (+18 to +25°C) for 5min.
2. Briefly centrifuge.
   Add **250µl buffer *DB1***.
   Add **10µl *MolDNase B*** (Kit 2), vortex for 15s.
   Incubate at room temperature (+18 to +25°C) for 15min.
3. Centrifuge at ≥12,000x*g*, 10min.
   Remove supernatant by pipetting and discard.
4. Resuspend pellet in **1ml buffer *RS*** by pipetting.
5. Centrifuge at ≥12,000x*g*, 5min.
   Remove supernatant by pipetting.
   (Optional: freeze pellet at -15 to -25°C for storage).
6. Resuspend pellet in **80µl buffer *RL***, briefly centrifuge tube.
7. Add **20µl *BugLysis*** (Kit 2).
   Add **1.4µl *ß-mercaptoethanol*** (Kit 2), vortex for 15s.
   Take care not to inhale.
   Incubate at 37°C, 30min, 1,000rpm (thermomixer).
8. Briefly centrifuge.
   Add **150µl buffer *RP***.
   Add **20µl *Proteinase K*** (Kit 2), vortex for 15s.
   Incubate at 56°C, 10 min, 1,000 rpm (thermomixer).

Removal of Human DNA

Lysis of Pathogens

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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*** (100µl each sample) vial to **70°C** (thermomixer).

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

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

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

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

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

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

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

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

Lysis of Pathogens

DNA Purification

DNA Elution

***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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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 2, short manual).

Per sample:

1. Add **250µl buffer *CM***, vortex for 15s.
   Let stand at room temperature (+18 to +25°C) for 5min.
2. Briefly centrifuge.
   Add **250µl buffer *DB1***.
   Add **10µl *MolDNase B*** (Kit 2), vortex for 15s.
   Incubate at room temperature (+18 to +25°C) for 15min.
3. Centrifuge at ≥12,000x*g*, 10min.
   Remove supernatant by pipetting and discard.
4. Resuspend pellet in **1ml buffer *RS*** by pipetting.
5. Centrifuge at ≥12,000x*g*, 5min.
   Remove supernatant by pipetting.
   (Optional: freeze pellet at -15 to -25°C for storage).
6. Resuspend pellet in **80µl buffer *RL***, briefly centrifuge tube.
   Add **20µl *BugLysis*** (Kit 2).
   Add **1.4µl *ß-mercaptoethanol*** (Kit 2), vortex for 15s.
   Take care not to inhale.
   Incubate at 37°C, 30min, 1,000rpm (thermomixer).
7. Briefly centrifuge.
   Add **150µl buffer *RP***.
   Add **20µl *Proteinase K*** (Kit 2), vortex for 15s.
   Incubate at 56°C, 10 min, 1,000 rpm (thermomixer).

Removal of Human DNA

Lysis of Pathogens

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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*** (100µl each sample) vial to **70°C** (thermomixer).

Lysis of Pathogens

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

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

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 60s.

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

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

DNA Elution

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

14. Add **100µl *Deionized Water*** heated to 70°C.
    Incubate at room temperature (+18 to +25°C) for 1min .
    Centrifuge: ≥12,000x*g*, 1min.
    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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