SepsiTest-UMD / Safety Information*

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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, reproductive 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 ***Enzyme 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+P312-P304+P340+P312-P305+P351+P338  **Warning**
Buffer CS	Contains guanidinium thiocyanate (> 10 %): **Acute toxicity (oral, skin), 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.** 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*
Buffer *WS*	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.

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 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; **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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SepsiTest-UMD / Sample Pre-Treatment*

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

- 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 Sample Tube (ST; Kit 1) in a rack, close the lid and mark with sample ID.

Fluid Sample Preparation

- For the preparation of blood samples use only K-EDTA or citrate-stabilised whole blood.

1. Pipette 1 ml of the stabilised blood into 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, package E) (use the measure line of the tube).

Continue with the instructions of the scheme SepsiTest-UMD / DNA Isolation (page 5).

Swab Sample Preparation

1. Pipette 1 ml of buffer *SU* (Kit 1, package E) into the *Sample tube* (*ST*). **Or:** If available in the swab vial, pipette 1 ml fluid into a *Sample tube* (*ST*) instead of buffer *SU*. In case of less sample volume available, fill up to 1 ml by pipetting buffer *SU* (use the measure line of the tube).
2. Wash the swab by swirling in the fluid of the *Sample tube* (*ST*) and pressing to the tube wall several times. Discard the swab.

Continue with the instructions of the scheme SepsiTest™-UMD / 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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SepsiTest-UMD / Sample Pre-Treatment*

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

- 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 Sample Tube (ST; Kit 1) in a rack, close the lid and mark with sample ID.

Tissue Sample Preparation

1. Pipette 180 µl of buffer *PKB* (Kit 1, package D) into the *Sample tube* (*ST*).
2. 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.
3. Transfer the cut specimen to the *Sample tube* (*ST*) filled with buffer *PKB.*
4. **Add 20 µl of *Enzyme K*** (Kit 2)**.**
5. **Vortex for 15 s** and **Incubate** at 56°C, 10 min, 1,000 rpm (thermomixer).
6. 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 SepsiTest-UMD / DNA Isolation (page 5).

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SepsiTest-UMD / DNA Isolation*

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

Unpack buffer vials (Kit 1, package A) and *Control DNA* (in Kit 2, bags), briefly centrifuge and place in a rack in the following order:

CM – DB1 – RS –RL – Control DNA – RP – CS – AB – WB –WS – ES

Continued from SepsiTest-UMD / Sample Pre-Treatment (pages 1 to 2).

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. Note: Preparation of this step **not** in the *ST* tube.
   Add **10 µl *Control DNA*** (in Kit 2, bags) into a vial **buffer *RP***, vortex for 15 s and briefly centrifuge. Continue to *ST* tube.
8. Briefly centrifuge. Add **150 µl buffer *RP* incl. *Control DNA***, Add **20 µl *Proteinase K*** (Kit 2), vortex for 15s. Incubate at 56°C, 10 min, 1,000 rpm (thermomixer). **Continue on page 6**

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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During 10 min incubation:

Kit 1 DNA Isolation Unit: Package C (Consumables)

Unpack *Spin columns* (*SC*), 2 ml *Collection tubes* (*CT*) and 1.5 ml *Elution tubes* (*ET*), label; heat **buffer *ES*** (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.*
    Tissue: Pipette the liquid phase in the column.
    Avoid transfer of any unresolved particles!
    Centrifuge: ≥12,000x*g*, 30 to 60 s.

12. Remove column and place in a new 2.0 ml *Collection tube.*
    Add **400 µl buffer *WB.***
    Centrifuge: ≥12,000x*g*, 30 to 60 s.

13. Remove column and place in a new 2.0 ml *Collection tube.*
    Add **400 µl *WS.***
    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 *ES*** 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 +4 to +12°C or for longer storage at -15 to -25°C.

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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

DNA-free

Before starting the mastermix preparation:

- **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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