SepsiTest ™-UMD / Safety Information*

Molzym

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%): **Poisonous, irritating, environ-mental hazardous** H227-H301-H310+H330-H315-H318-H410; P273-P301+P310-P302+P352-P304+P340-P305+P351+P338	**Danger**
Proteinase K Enzyme K	Contains *Proteinase K* (≥1%): **Respiratory sensitization, irritating (eyes and skin) and specific target organ toxicity – single exposure** H315-H319-H334-H335; P302+P352-P304+P341-P305+P351+P338	**Danger**
Buffer CS	Contains guanidinium thiocyanate (>10%): **Acute toxicity (oral, dermal, inhalation) and chronic aquatic toxicity** H302-H312-H332-H412-EUH032; P260-P273-P301+P312-P302+P350-P304+P340	**Warning**
Buffer *AB*	Contains 2-propanol (<40%): **Flammable liquids and irritating (eyes)** H225-H319-H336; P210-P233-P304+P340-P305+P351+P338	**Danger**
Buffer *WB*	Contains isopropanol (≥40%): **Flammable liquids and irritating (eyes)** H225-H319-H336; P210-P233-P304+P340-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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Version 03

Safety Information for Sample Pre-Treatment and DNA Isolation

Component	Classification and Hazard / Precautionary Statements*
Buffer *WS*	Contains ethanol (>50%) **Flammable liquids** H225; P210-P233 **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: Poison Information Center Mainz, **Germany,** Tel.: +49-6131-19240 / 24h (German, English, French). Outside of Germany: Please contact the regional company representation in your country.

* **H225**: Highly flammable liquid and vapour; **H227**: Combustible liquids; **H301**: Toxic if swallowed; **H302:** Harmful if swallowed; **H302+312+332:** Harmful if swallowed, in contact with skin or if inhaled; **H310+H330**: Fatal in contact with skin or if inhaled; **H311+H331**: Toxic in contact with skin or if inhaled; **H312**: Harmful in contact with skin; **H315**: Causes skin irritation; **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; **H335**: May cause respiratory irritation; **H336**: May cause drowsiness or dizziness; **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/sparks/open flames/hot surfaces. – No-smoking; **P233**: Keep container tightly closed; **P260**: Do not breath fume/gas/mist/vapours; **P273:** Avoid release to the environment; **P301+P310:** IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician; **P301+P312:** IF SWALLOWED: Rinse mouth. Do NOT induce vomiting; **P302+P350**: IF ON SKIN: Gently wash with plenty of soap and water; **P302+P352:** IF ON SKIN: Wash with plenty of soap and water; **P304+P340:** IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing;**P304+P341**: IF INHALED: If breathing difficult, remove to fresh air and keep at rest in a position comfortable for breathing; **P305+P351+P338:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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Version 03

- 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

Kit 1 DNA Isolation Unit: Buffers and Consumables (packages B and E)

- For the preparation of blood samples use only K-EDTA or citrate-stabilised whole blood.
- Place a *Sample tube* (*ST*; Kit 1, package B) in a rack and mark.
- Pipette 1ml 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 1ml 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).

Fluid

Swab Sample Preparation

Kit 1 DNA Isolation Unit: Buffers and Consumables (packages B and E)

- Place a *Sample tube* (*ST*; Kit 1, package B) in a rack and mark.
- Pipette 1ml of buffer *SU* (Kit 1, package E) into the *Sample tube* (*ST*)
- **Or:** If available in the swab vial, pipette 1ml fluid into a *Sample tube* (*ST*) instead of buffer *SU*. In case of less sample volume available, fill up to 1ml by pipetting buffer *SU* (use the measure line of the tube).
- 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).

Swab

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- Work under a laminar flow, Class II biological safety cabinet.
- Transport the sample under conditions avoiding contamination to the laboratory for analysis.

Tissue Sample Preparation

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

Tissue

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

- Per specimen, place a *Sample tube* (*ST*, Kit 1, package B) in a rack and mark. Pipette 180µl of buffer *PKB* (Kit 1, package D) 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 *Enzyme 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 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.**

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:

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. Add **10µl *Control DNA*** (in Kit 2, bags) into a vial **buffer *RP***, vortex for 15s and briefly centrifuge.
8. Briefly centrifuge. Add **150µl buffer *RP* incl. *Control DNA***, Add **20µl *Proteinase K*** (Kit 2), vortex for 15s. Incubate at 56°C, 10 min, 1,000 rpm (thermomixer).

Depletion of Human DNA

Lysis of Pathogens

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.**

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*** (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.*
    Tissue: Pipette the liquid phase in the column.
    Avoid transfer of any unresolved particles!
    Centrifuge: ≥12,000x*g*, 30 to 60s.

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

13. Remove column and place in a new 2.0ml *Collection tube.*
    Add **400µl *WS.***
    Centrifuge: ≥12,000x*g*, 3min.

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

15. Add **100µl *ES*** 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 +4 to +12°C or for longer storage at -15 to -25°C.

Lysis of Pathogens

DNA Purification

DNA Elution

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Places where Handlings are performed

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* (998µl DNA dilution buffer + 2µl *P1*)

PCR Assaying

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

DNA-free

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**)**

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Note: Keep all PCR tubes filled with **PCR-ready mastermixes** and the ***MolTaq 16S*** 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

- **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*
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:

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

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

DNA

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

- **Start the PCR programme of Eppendorf Mastercycler** (manual Tab.5, page 30)

  See pages 35 to 37 of the manual for other cyclers.

Preparation of Mastermixes *MA Bac, MA Yeasts or MA Control*

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