Add-On 10

To be used with
SepsiTest™-UMD and UMD-SelectNA™

Extraction of bacterial and fungal DNA from 1 to 10ml whole blood and other primary sterile body liquids

Medium Size Protocol (>1 to 5ml)

Large Size Protocol (>5 to 10ml)

CE IVD

- For *in-vitro* diagnostic use -

Molzym

Version 3.1

Date of first release: 06/2014
Last update: 07/2017

Contents

page

Kit Information

Kit Contents – *Add-On 10*

	24 rxn	48 rxn
DNA Isolation		
A) Extraction Buffers, in bottles		
SU	1x 125ml	2x 125ml
CM	1x 100ml	2x 100ml
DB1	1x 100ml	2x 100ml
B) Consumables, in bags		
50ml Centrifuge Tubes	1x 25	1x 50
Manuals		
Manual	1x	1x
Short manual	2x	2x

Symbols

Symbols used in labelling and in section 'Hazard and Precautionary Statements' (page 4).

For *in-vitro* diagnostic use


Manufactured by

Consult instructions for use


Irritant


This product fulfils the requirements of the European Directive 98/79 EC for *in-vitro* diagnostic medical devices.


Use by

Batch code


Content of the package


Temperature limitation (store at)

Catalogue number

Storage and Stability

Guarantee for ***full performance*** of buffers and consumables is given for ***24 months*** at the conditions specified and is guaranteed only if ***packed material*** is ***undamaged*** upon arrival. Once opened, the vials have to be used as specified by the protocol.

Buffers and consumables of the ***Add-On 10*** kit should be stored dry in the dark and at room temperature (+18 to +25°C). Opened bottles/consumables can be stored at a dark place at room temperature (+18 to +25°C).

Product Use Limitations

Add-On 10 is intended as a kit for for ***in-vitro*** **diagnostic use with whole blood and other primary sterile body liquid samples**. ***Add-On 10*** is not claimed or intended to be used for the detection and identification of any specific pathogen or not for clinical use of other specimens than specified above, including diagnostic, therapeutic or blood banking. The CE marking is limited to the detection of bacteria and fungi without further taxonomic differentiation at the detection level. ***Add-On 10*** is not intended to be used as the only diagnostic tool for the presence of bacteria and fungi in the specimens, but rather as a means of rapid detection of pathogens flanking standard culturing analysis. It is emphasised that, provided all controls are as expected, positive results should be confirmed by sequencing analysis. Sample analysis may bear the possibility of detecting skin colonisers contaminating samples.

This Add-on kit is only to be used in combination with the following kits: *SepsiTest™-UMD* or *UMD-SelectNA™*!

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable sleeve covers, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS) which are available on request.

CAUTION: Never add hypochlorite (bleach) or acidic solutions directly to the sample-preparation waste.

Buffer *CM* contains guanidine hydrochloride, which can form highly reactive compounds and toxic gases when combined with hypochlorite or other acidic solutions. If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area with 70% (v/v) ethanol. This kit is to be used only by skilled personnel trained for handling infectious material and molecular biological methods. To avoid false analytic results by DNA contamination of reagents and infection of the user by infectious agents during handling, always wear sterile protective gloves, sterile disposable sleeve covers, a lab coat, protective goggles and disposable overshoes. Work in a Class II biological safety cabinet irradiated with UV before starting according to the instruction manual of the manufacturer. The UV lamp must be switched off during working. Follow the instructions of the manufacturer for maintenance of the workstation. Dispose potentially infectious material and the waste of the sample preparation according to the national directive of the health organisation (e.g., Richtlinie über die ordnungsgemäße Entsorgung von Abfällen aus Einrichtungen des Gesundheitsdienstes, 2002).

Molzym reserves the right to alter or modify the product to improve the performance of the kit.

Hazard and Precautionary Statements

Buffer *CM*
Contains guanidine hydrochloride (>10%):
Acute toxicity (oral) and irritating (eyes and skin).

Warning

Hazard and precautionary statements*:
H302-H315-H319; P301+P312-P302+P352-P305+P351+P338

Emergency information (24-hours service)
Emergency medical information in English, French, and German can be obtained 24 hours a day from: Poison Information Centre Mainz, Germany; Tel: +49(0)6131 19 24 0
Outside of Germany: Please contact the regional company representation in your country.

* **H302:** Harmful if swallowed; **H315**: Causes skin irritation; **H319**: Causes serious eye irritation.

P301+P312: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting; **P302+P352:** IF ON SKIN: Wash with plenty of soap and water; **P305+P351+P338:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

System Description

A detailed description of the test principle is described in the manuals of the ***SepsiTest™-UMD*** or ***UMD-SelectNA™*** kits.

Add-On 10 allows the isolation of pathogen DNA from medium (>1 to 5ml) and large volumes (>5 to 10ml) of whole blood and other primary sterile body liquids in combination with the kit ***SepsiTest™-UMD*** and the automated system ***UMD-SelectNA™***. The use of ***Add-On 10*** allows an increased analytical sensitivity (Tab. 1).

Performance

The ***Add-On 10*** kit has been evaluated using a range of volumes of EDTA blood samples spiked with defined loads of strains (Tab. 1).

The assays used were, *MA Bac* (bacteria) and *MA Yeasts* (fungi) of the ***SepsiTest™-UMD*** and ***UMD-SelectNA™*** kits.

The analytical system for the detection of a methicillin-resistant *Staphylococcus aureus* strain was the FluoroType® MRSA test (Hain Lifescience, Nehren, Germany).

For instance, in mock culture experiments, *Staphylococcus aureus* and *Escherichia coli* were detected at <60cfu/ml (1ml), 30cfu/ml (2ml), 12cfu/ml (5ml) and <6cfu/ml (10ml) and at 145cfu/ml (1ml), 36cfu/ml (2ml), 14cfu/ml (5ml) and 7cfu/ml (10ml), respectively. Other examples are shown in Tab. 1.

Tab. 1: Detection of microbial DNA (PCR assays *MA Bac* and *MA Yeasts*) in mock blood samples extracted with ***SepsiTest™-UMD*** (1ml) and ***Add-On 10*** plus ***SepsiTest™-UMD*** or ***UMD-SelectNA™*** (10ml). [n = 3].

Strain	Analytical Systems [a)]	Limit of detection [cfu/ml]	
		SepsiTest™-UMD 1ml blood sample	Add-On 10 + SepsiTest™-UMD 10ml blood sample
Staphylococcus aureus (strain 1), Methicillin-sensitive	*MA Bac*	<60 [b)]	<6 [b)]
Staphylococcus aureus (strain 2), Methicillin-sensitive	*MA Bac*	20	1
Staphylococcus aureus, Methicillin-resistant	FluoroType® MRSA	<50	<5
Escherichia coli	*MA Bac*	145 [b)]	7 [b)]
Candida albicans	*MA Yeasts*	10	1

[a)] **Analytical systems:** *MA Bac* and *MA Yeasts* (SepsiTest™-UMD PCR assays, Molzym); FluoroType® MRSA (Hain Lifescience, Nehren, Germany).

[b)] **UMD-SelectNA™** (automated system)

Protocols

Add-on procedure for up to 10ml whole blood and other primary sterile body liquids in combination with the kits ***SepsiTest™-UMD*** (part 1, pages 6 to 8) or ***UMD-SelectNA™*** (part 2, pages 9 to 11).

Part 1: Add-on Protocols for *SepsiTest™-UMD*

Additional Information

Please consult the manual of the ***SepsiTest™-UMD*** kit for the following information:

- **Avoidance of DNA Contamination** (***SepsiTest™-UMD*** manual, page 17)
- **Sample collection** (***SepsiTest™-UMD*** manual, page 19)
- **Isolation of Pathogen DNA** (***SepsiTest™-UMD*** manual, pages 19 to 20)
- **Apparatuses and Consumables to be Supplied by the User** (***SepsiTest™-UMD*** manual, pages 6 to 7)

! In addition, the following items are needed to perform the ***Add-On 10*** protocol:

- A high speed centrifuge (9,500x*g*) equipped with a fixed angle rotor for the *50ml Centrifuge Tubes* (supplied)
- Sterile, disposable 5ml pipettes with aerosol filter, or a 5ml tip of a precision pipette.

Procedure: Sample Pre-Treatment for Human DNA Removal

Caution: Wear sterile protective gloves, sterile disposable sleeve covers, a disposable lab coat and protective goggles when handling infectious material. Work in a Class II biological safety cabinet irradiated with UV before starting according to the instructions of the manufacturer. Use fresh pipette tips with each pipetting step.

How to Start

- ***Add-On 10*** (store kit at +18 to +25°C)
  The kit contains bottles of buffers (*SU*, *CM* and *DB1*) and *50ml Centrifuge Tubes*.

  Buffers and consumables of the ***Add-On 10*** have to be stored at room temperature (+18 to +25°C) in a dark, DNA-free place until the expiration date of the kit. Do not place the buffers in a refrigerator, because this would result in precipitation and loss of function of the buffers!

The following components of the ***SepsiTest™-UMD*** kit are needed:

- **Kit 1** (store at +18 to +25°C)

  The kit contains buffer *RS* (package A) and *Sample tubes* (*ST* tubes, package B).

- **Kit 2** (store at -15 to -25°C)

  The kit contains *MolDNase B*.

Note: Please read the information (part 1 'Sample Collection' [***SepsiTest™-UMD*** manual, page 19], 'Isolation of Pathogen DNA' [***SepsiTest™-UMD*** manual, pages 19 to 20] and 'How to Start' [***SepsiTest™-UMD*** manual, page 20]) **before starting the procedure!**

Protocol 1: Medium Volume Samples
(>1 to 5ml Blood and Other Primary Sterile Body Liquids)

A) Fill up procedure for samples less than 5ml volume

Samples >1ml and less than 5ml are filled up using buffer *SU* (***Add-On 10***). Transfer the sample by pipetting into a *50ml Centrifuge Tube* (***Add-On 10***). Then add buffer *SU* using a disposable 5ml pipette or pipette tip until reaching the 5ml mark of the tube. Discard pipette/pipette tip with excess buffer *SU*. Continue with part B (below).

B) Sample pre-treatment and DNA Isolation

1. **Pipette 5ml sample or use filled-up sample (part A, above) into a *50ml Centrifuge Tube* (*Add-On 10*).**

   Add 2ml buffer *CM* (*Add-On 10*). Vortex at full speed for 15s. Let stand on the bench at room temperature (+18 to +25°C) for 5min.

   Buffer *CM* is a chaotropic buffer that lyses the human cells. For optimal results it is important to mix thoroughly.

   Caution: Buffer *CM* is an irritant. Avoid contact with skin and eyes.

2. **Add 2ml buffer *DB1* (*Add-On 10*) and 10µl *MolDNase B* (*SepsiTest™-UMD,* Kit 2) to the lysate and immediately vortex for 15s. Let stand on the bench for 15min.**

   During this step the DNA released from human cells is degraded.

3. **Centrifuge the *50ml Centrifuge Tube* in a high speed centrifuge at 9,500x*g* for 10min. Thereafter, carefully decant the supernatant.**

4. **Add 1ml buffer *RS* (*SepsiTest™-UMD,* Kit 1) and resuspend the sediment by pipetting in and out. Transfer the suspension by pipetting into a *Sample tube* (*ST* tubes, *SepsiTest™-UMD,* Kit 1).**

   The sediment consists of cell debris and pathogen cells. Resuspension may take some time. Take care that all visible material has been resuspended.

Continue with the instructions of the ***SepsiTest™-UMD*** manual, part 1C, page 22, step 5.

Protocol 2: Large Volume Samples
(>5 to 10ml Blood and Other Primary Sterile Body Liquids)

A) Fill up procedure for samples less than 10ml volume

Samples >5ml and less than 10ml are filled up using buffer *SU* (***Add-On 10***). Transfer the sample by pipetting into a sterile *50ml Centrifuge Tube* (***Add-On 10***). Then add buffer *SU* using a disposable 5ml pipette or pipette tip until reaching the 10ml mark of the tube. Discard pipette/pipette tip with excess buffer *SU*. Continue with part B (below).

B) Sample pre-treatment and DNA Isolation

1. **Pipette 10ml sample or use filled-up sample (part A, above) into a *50ml Centrifuge Tube* (*Add-On 10*).**

   Add 4ml buffer *CM* (*Add-On 10*). Vortex at full speed for 15s. Let stand on the bench at room temperature (+18 to +25°C) for 5min.

   Buffer *CM* is a chaotropic buffer that lyses the human cells. For optimal results it is important to mix thoroughly.

   Caution: Buffer *CM* is an irritant. Avoid contact with skin and eyes.

2. **Add 4ml buffer *DB1* (*Add-On 10*) and 10µl *MolDNase B* (*SepsiTest™-UMD,* Kit 2*) to the lysate and immediately vortex for 15s. Let stand on the bench for 15min.**

   During this step the DNA released from human cells is degraded.

3. **Centrifuge the *50ml Centrifuge Tube* in a high speed centrifuge at 9,500x*g* for 10min. Thereafter, carefully decant the supernatant.**

4. **Add 1ml buffer *RS* (*SepsiTest™-UMD,* Kit 1) and resuspend the sediment by pipetting in and out. Transfer the suspension by pipetting into a *Sample tube* (*ST* tubes, *SepsiTest™-UMD,* Kit 1).**

   The sediment consists of cell debris and pathogen cells. Resuspension may take some time. Take care that all visible material has been resuspended.

Continue with the instructions of the manual ***SepsiTest™-UMD***, part 1C, page 22, step 5.

Part 2: Add-on Protocols for *UMD-SelectNA™*

Additional Information

Please consult the manual of the ***UMD-SelectNA™*** kit for the following information:

- **Avoidance of DNA Contamination** (***UMD-SelectNA™*** manual, page 17)
- **Sample collection** (***UMD-SelectNA™*** manual, page 19)
- **Isolation of Pathogen DNA** (***UMD-SelectNA™*** manual, pages 19 to 20)
- **Apparatuses and Consumables to be Supplied by the User** (***UMD-SelectNA™*** manual, pages 6 to 7)

! In addition, the following items are needed to perform the ***Add-On 10*** protocol:

- A high speed centrifuge (9,500x*g*) equipped with a fixed angle rotor for the *50ml Centrifuge Tubes* (supplied)
- Sterile, disposable 5ml pipettes with aerosol filter, or a 5ml tip of a precision pipette.

Procedure: Sample Pre-Treatment for Human DNA Removal

Caution: Wear sterile protective gloves, sterile disposable sleeve covers, a disposable lab coat and protective goggles when handling infectious material. Work in a Class II biological safety cabinet irradiated with UV before starting according to the instructions of the manufacturer. Use fresh pipette tips with each pipetting step.

How to Start

- ***Add-On 10*** (store kit at +18 to +25°C)
  The kit contains bottles of buffers (*SU*, *CM* and *DB1*) and *50ml Centrifuge Tubes*.

  Buffers and consumables of the ***Add-On 10*** have to be stored at room temperature (+18 to +25°C) in a dark, DNA-free place until the expiration date of the kit. Do not place the buffers in a refrigerator, because this would result in precipitation and loss of function of the buffers!

The following components of the ***UMD-SelectNA™*** kit are needed:

- **Kit 1** (store at +18 to +25°C)
  The kit contains buffer *RS* and *Sample tubes* (*ST* tubes).

- **Kit 2** (store at -15 to -25°C)
  The kit contains *MolDNase B*.

Note: Please read the information (part 1 'Sample Collection' [***UMD-SelectNA™*** manual, page 19], 'Isolation of Pathogen DNA' [***UMD-SelectNA™*** manual, pages 19 to 20] and 'How to Start' [***UMD-SelectNA™*** manual, page 20]) **before starting the procedure!**

Protocol 1: Medium Volume Samples
(>1 to 5ml Blood and Other Primary Sterile Body Liquids)

A) Fill up procedure for samples less than 5ml volume

Samples >1ml and less than 5ml are filled up using buffer *SU* (***Add-On 10***). Transfer the sample by pipetting into a *50ml Centrifuge Tube* (***Add-On 10***). Then add buffer *SU* using a disposable 5ml pipette or pipette tip until reaching the 5ml mark of the tube. Discard pipette/pipette tip with excess buffer *SU*. Continue with part B (below).

B) Sample pre-treatment and DNA Isolation

1. **Pipette 5ml sample or use filled-up sample (part A, above) into a *50ml Centrifuge Tube* (*Add-On 10*).**

   Add 2ml buffer *CM* (*Add-On 10*). Vortex at full speed for 15s. Let stand on the bench at room temperature (+18 to +25°C) for 5min.

   Buffer *CM* is a chaotropic buffer that lyses the human cells. For optimal results it is important to mix thoroughly.

   Caution: Buffer *CM* is an irritant. Avoid contact with skin and eyes.

2. **Add 2ml buffer *DB1* (*Add-On 10*) and 10µl *MolDNase B* (*UMD-SelectNA™*, Kit 2) to the lysate and immediately vortex for 15s. Let stand on the bench for 15min.**

   During this step the DNA released from human cells is degraded.

3. **Centrifuge the *50ml Centrifuge Tube* in a high speed centrifuge at 9,500x*g* for 10min. Thereafter, carefully decant the supernatant.**

4. **Add 1ml buffer *RS* (*UMD-SelectNA™*, Kit 1) and resuspend the sediment by pipetting in and out. Transfer the suspension by pipetting into a *Sample tube* (*ST* tubes, *UMD-SelectNA™*, Kit 1).**

   The sediment consists of cell debris and pathogen cells. Resuspension may take some time. Take care that all visible material has been resuspended.

Continue with the instructions of the ***UMD-SelectNA™*** manual, part 1C, page 22, step 5.

Protocol 2: Large Volume Samples
(>5 to 10ml Blood and Other Primary Sterile Body Liquids)

A) Fill up procedure for samples less than 10ml volume

Samples >5ml and less than 10ml are filled up using buffer *SU* (***Add-On 10***). Transfer the sample by pipetting into a sterile *50ml Centrifuge Tube* (***Add-On 10***). Then add buffer *SU* using a disposable 5ml pipette or pipette tip until reaching the 10ml mark of the tube. Discard pipette/pipette tip with excess buffer *SU*. Continue with part B (below).

B) Sample pre-treatment and DNA Isolation

1. **Pipette 10ml sample or use filled-up sample (part A, above) into a *50ml Centrifuge Tube* (*Add-On 10*).**

   Add 4ml buffer *CM* (*Add-On 10*). Vortex at full speed for 15s. Let stand on the bench at room temperature (+18 to +25°C) for 5min.

   Buffer *CM* is a chaotropic buffer that lyses the human cells. For optimal results it is important to mix thoroughly.

   Caution: Buffer *CM* is an irritant. Avoid contact with skin and eyes.

2. **Add 4ml buffer *DB1* (*Add-On 10*) and 10µl *MolDNase B* (*UMD-SelectNA™*, Kit 2*)* to the lysate and immediately vortex for 15s. Let stand on the bench for 15min.**

   During this step the DNA released from human cells is degraded.

3. **Centrifuge the *50ml Centrifuge Tube* in a high speed centrifuge at 9,500x*g* for 10min. Thereafter, carefully decant the supernatant.**

4. **Add 1ml buffer *RS* (*UMD-SelectNA™*, Kit 1) and resuspend the sediment by pipetting in and out. Transfer the suspension by pipetting into a *Sample tube* (*ST* tubes, *UMD-SelectNA™*, Kit 1).**

   The sediment consists of cell debris and pathogen cells. Resuspension may take some time. Take care that all visible material has been resuspended.

Continue with the instructions of the ***UMD-SelectNA™*** manual, part 1C, page 22, step 5.

Supplementary Information

Troubleshooting

See 'Troubleshooting' of the ***SepsiTest™-UMD*** (page 43) or ***UMD-SelectNA™*** (page 47) manual.

References

See 'References' of the ***SepsiTest™-UMD*** (pages 44 to 46) or ***UMD-SelectNA™*** (pages 48 to 50) manual.

Tradenames

Add-On 10, ***SepsiTest™-UMD***, ***UMD-SelectNA™*** are trade names of Molzym.

Technical Support

If you have any questions please contact us.
Our hotline: +49(0)421 69 61 62 0
E-Mail: support@molzym.com • **Web:** www.molzym.com

Material safety data sheets are available on request.

Order Information

Product	Contents and Application	Cat. No.
Add-On 10 (CE-IVD)	Add on kit for ***SepsiTest™-UMD*** or ***UMD-SelectNA™***. Body liquid samples >1 to 10ml	
	24 sample tests	U-120-024
	48 sample tests	U-120-048
Products for the use of the *Add-On 10* kit:		
SepsiTest™-UMD (CE-IVD)	Pathogen DNA isolation for **body liquids**, swabs and tissues and PCR detection.	
	24 sample tests	U-010-024
	48 sample tests	U-010-048
UMD-SelectNA™ (CE-IVD)	Automated pathogen DNA extraction and PCR analyses from **body liquids**, swabs and tissue	
	24 sample tests	U-050-024
	48 sample tests	U-050-048

Order Hotline:

Tel.: +49(0)421 69 61 62 0 • **Fax:** +49(0)421 69 61 62 11 • **E-Mail:** order@molzym.com

Contact

Molzym GmbH & Co. KG
Mary-Astell Str. 10
28359 Bremen, Germany

Tel.: +49(0)421 69 61 62 0 • **Fax:** +49(0)421 69 61 62 11
E-Mail: info@molzym.com • **Web:** www.molzym.com, www.sepsitest.de