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Microbiological Assay

REF MSA01

100 tests

Sample Pretreatment Reagent

Sample pretreatment reagent is a reagent which used for pretreatment of bacteria and fungi in the identification of measurand for using with MALDI-TOF MS.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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CE IVD

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Introduction

In the clinical microbiology laboratories, microbial identification is conventionally done by phenotypic and biochemical analysis mostly using automated systems. They are required time ranging from a few hours to several days depending on microbial species. MALDI-TOF MS technology makes generation of unique mass spectral fingerprints of microorganisms possible, which are mostly a snapshot of ribosomal proteins ideal for an accurate microbial identification at the species level.[1] MALDI-TOF MS can rapidly and accurately identify a wide range of microorganisms at a reasonable cost using only a portion or the entire colony and a drop of matrix solution.[2, 3] The ability of MALDI-TOF MS to directly identify bacteria in positive blood cultures is also important for the effective management of bloodstream infections.[4, 5]

Measurement Principle

The sample pretreatment reagent is used for pretreatment of bacteria and fungi in the identification of measurand for use with MALDI-TOF MS. Lysate 1 and Lysate 2 are used to destroy the cell wall of the sample and extract the ribosomal protein. 1 μL of the matrix solution is added to the spot with the extracted protein to generate the crystal. Perform the identification in accordance with the instruction of MALDI-TOF MS.

Components

1. Lysate 1

1 vial containing 1.0 mL solution with formic acid.

2. Lysate 2

1 vial containing 1.1 mL solution with acetonitrile.

3. Buffer

1 vial containing 0.3 mL solution with trifluoroacetic acid.

4. Matrix

1 vial containing 2.0 mg α-cyano-4-hydroxy-cinnamic acid.

Materials Required but not Provided

1. Distilled water
2. Centrifuge
3. Centrifuge tube
4. Micropipette
5. Ethyl alcohol absolute

Assay analyzers on which the reagent can be used

- Autof ms (Autobio)
- Microflex LT/SH (Bruker)

The Sample Pretreatment Reagent is intended for use on MALDI-TOF MS which is Autof ms (Autobio) or Microflex LT/SH (Bruker).

Warnings and Precautions

Health and safety information

For Lysate 1, which contains formic acid, the following statements apply.

GHS 05
Danger

H314 Causes severe skin burns and eye damage.
P260 Do not breathe dusts or mists.
P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER/doctor.
P321 Specific treatment (see on this label).
P405 Store locked up.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

For Lysate 2, which contains acetonitrile, the following statements apply.

GHS 02
Danger

GHS 07
Warning

H225 Highly flammable liquid and vapour.
H302+H312+H332 Harmful if swallowed, in contact with skin or if inhaled.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, open flames and other ignition sources. No smoking.
P241 Use explosion-proof [electrical/ventilating/lighting] equipment.
P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P403+P235 Store in a well-ventilated place. Keep cool.
P501 Dispose of contens/container in accordance with local/regional/national/international regulations.

For Buffer, which contains trifluoroacetic acid, the following statements apply.

GHS 05
Danger

H314 Causes severe skin burns and eye damage.
P260 Do not breathe dusts or mists.
P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER/doctor.
P321 Specific treatment (see on this label).
P405 Store locked up.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Handle the potentially contaminated materials and wastes safely according to local requirement.
4. Do not smoke, drink, eat or use cosmetics in the working area. Keep the reagents away from fire.
5. Wear protective clothing and disposable gloves when dealing with the Lysate and Buffer. Wash hands after operations.
6. Conduct the dilution procedure away from bad ambient conditions. e. g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
7. Do not use reagent beyond the labeled expiry date. Store the remaining reagent at 2-8 ℃, and be certain the lid is securely sealed.
8. Do not mix or substitute solution from other manufacturers.
9. The presence of a small amount of yellow precipitation in prepared matrix solution is normal, mix gently before use.

10. Consider the samples and reagents as potentially infectious material and deal them in accordance with local requirement.
11. The reagents have chemical hazard, avoid contact to skin or mucosa. If happened as follows:
    Skin or mucosa contact: Take off contaminated clothes, wash the area extensively with water, and seek for medical treatment if necessary.
12. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
13. When any damage to the protective packaging or any change of dissolubility or usage characteristic is observed, do not use the kit.
14. Avoid the culture medium when picking out the colony.
15. Mix the sample sufficiently with the reagents during the pretreatment process.
16. Avoid cross-contamination when using the micropipette.
17. Calibrate the MALDI-TOF MS according to the instructions of the analyzer before the identification to avoid the deviation of the result due to the analyzer.

Storage

1. Store all components at 2-8 ℃. Do not freeze. When stored as direction, all components are stable until the expiration date. Avoid strong light.
2. Seal the prepared matrix solution at 2-8 ℃, under which conditions the stability will be retained for 30 days.

Sample

1. Select fresh isolated colonies from patient sample source. For bacteria, culture the sample at appropriate temperature for 24 hours to obtain isolated colonies; for fungi, culture the sample at appropriate temperature for 36-48 hours to obtain isolated colonies.
2. After pretreatment, the sample shall be identified within 2 hours.

Pretreatment Procedure

1. Check the consumable materials
- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the MALDI-TOF MS's operation manual.

2. Reagent preparation
- Add 100 μL Lysate 2 and 100 μL Buffer to the Matrix. Shake and vortex until completely dissolved. The prepared matrix solution is clear yellow liquid.

3. Pretreatment tests
- Add 300 μL distilled water to the centrifuge tube. Inoculate 1-2 isolated colonies to the centrifuge tube, shake completely and mix well. Then add 900 μL ethyl alcohol absolute to the centrifuge tube and mix well.
- Centrifuge the dissolved sample with 8000-14800 rpm for 2-4 minutes, decant the supernatant. Dry the sediment at 37-40℃ for 2-5 minutes until the surface has no obvious water mark.
- Add 10 μL Lysate 1, mix well.
- Add 10 μL Lysate 2, mix well.
- Centrifuge the dissolved sample with 8000-14800rpm for 2-4 minutes.
- Inoculate 1 μL supernatant to the target slide, dry the sample spot without obvious water mark.
- Overlay the sample spot with 1 μL matrix solution, dry the sample spot without obvious water mark. Then identify the sample with MALDI-TOF MS.

Measurement Results

1. Analyse the identification results of microorganism according to the analysis method of microbial mass spectrometry.
2. Construct the database according to the analysis method of microbial mass spectrometry.

Limitations of the Procedure

1. The product can't be used alone and should be used together with MALDI-TOF MS.
2. If the sample count is too little (less than the sensitivity of the MALDI-TOF MS), the result is not reportable.
3. The test results cannot be effectively improved by using this kit because of the limitations of the MALDI-TOF MS.

Control Procedure

The recommended control procedure for this assay is to identify the reference strains *E. Coli* ATCC®PTA-1977 and *Candida albicans* ATCC®10231 separately. Conduct the same operations as in **Pretreatment Procedure** on the two strains. The result is valid if the results are *E. Coli* and *Candida albicans,* and confirmed to the species level.

Performance Characteristics

1. Dissolubility

Add 100 μL Lysate 2 and 100 μL Buffer to the Matrix. Shake and vortex until completely dissolved. It is normal to have trace of precipitation at the bottom.

Literature References

1. A. Croxatto, G. Prod'hom, and G. Greub, "Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology," *FEMS Microbiology Reviews*, vol. 36, no. 2, pp. 380-407, 2012.
2. M.A. Claydon, S.N. Davey, V. Edwards-Jines, and D. B. Gordon, "The rapid identification of intact microorganisms using mass spectrometry," *Nature Biotechnology*, vol. 14, no. 11, pp. 1584-1586, 1996.
3. R. D. Holland, J. G. Wilkes, F. Rafii et al., "Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 10, no. 10, pp. 1227-1232, 1996.
4. M. Drancourt, "Detection of microorganisms in blood specimens using matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a review," *Clinical Microbiology and Infection*, vol. 16, no. 11, pp. 1620-1625, 2010.
5. Y. Hoyos-Mallecot, C. Riazzo, C. Miranda-Casas, M. Rojo Martín, J. Gutiérrez-Fernández, and J. Navarro-Marí, "Rapid detection and identification of strains carrying carbapenemasses directly from positive blood culture using MALDI-TOF MS," *Journal of Microbiological Method*, vol. 105, pp. 98-101, 2014.