INSTRUCTIONS FOR USE

MUCAP TEST

1 - INTENDED USE
In vitro diagnostic. Liquid reagent for rapid differentiation of Salmonella spp. colonies directly on selective enteric plating media.

2 - COMPOSITION – BOTTLE CONTENT
4- methylumbellyferil caprylate dissolved in heptane   8 mL

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Gastrointestinal infections are still a global public health problem, being a leading cause of medical consultation. Among the bacteria responsible for these syndromes, Salmonella spp. are one of the most common causes of bacterial diarrheal diseases. The fluorogenic substrates, derived from methylumbe lliferone and coumarins, have been widely tested for the rapid identification of various microbial species and have demonstrated high sensitivity.1,2 The MUCAP Test reagent, developed and synthesized by Biolife and earlier described in some scientific papers,3,4 is an easy and rapid screening test for presumptive identification of Salmonella colonies, directly on selective enteric plating media,5,6 using an eight-carbon-atom ester conjugated with methylumbelliferone. The MUCAP Test reagent detects the C8 esterase enzyme, encoded by the apeE gene, present in Salmonella spp.9 The MUCAP Test reagent consists of 4-methylumbellyferil caprylate dissolved in heptane; this substrate interacts with the Salmonella C8 esterase in a specific manner, leading to the rapid release on the medium of umbelliferone, strongly fluorescent under Wood's lamp at 366 nm.

4 - PHYSICAL CHARACTERISTICS
Reagent appearance   colourless, limpid

5 - MATERIALS PROVIDED - PACKAGING

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<thead>
<tr>
<th>Product</th>
<th>Type</th>
<th>REF</th>
<th>Pack</th>
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<tbody>
<tr>
<td>MUCAP Test</td>
<td>Identification reagent</td>
<td>191500</td>
<td>8 mL (160 tests)</td>
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<td>Primary packaging: glass bottle with dropper</td>
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<td>Secondary packaging: cardboard box</td>
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6 - MATERIALS REQUIRED BUT NOT PROVIDED
Wood's lamp (366 nm), ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS
In clinical and non-clinical microbiology, the specimens consist of suspected Salmonella colonies grown on selective enteric plating media. MUCAP Test reagent cannot be used for the direct testing of clinical specimens.

8 - TEST PROCEDURE
The test is carried-out by flooding with one drop of MUCAP reagent all the suspect Salmonella colonies (lactose negative and H2S positive or negative colonies) cultivated on a selective enteric plating medium. The following scheme should be used:
1. Observe the colonies under a Wood's lamp (366nm) before adding the reagent to ensure that no spontaneous fluorescence occurs.
2. Add a drop of reagent to each isolated colony or to a group of colonies.
3. After 3-5 minutes observe the plates under the Wood's lamp (wavelength 366 nm) in semi-darkness.

9 - READING AND INTERPRETATION
Positive result: appearance of a blue fluorescence over the whole colony or on the edge of black centred colonies. Fluorescence negative colonies can be presumptively identified as Salmonella and subjected to a complete identification with appropriate tests. Fluorescence negative colonies can be considered non-Salmonella and therefore plates are discarded.

10 - USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed the test strains useful for the quality control.
Positive control: S.Typhimurium ATCC 14028
Negative control: P.mirabilis ATCC 25933

ATCC is a trademark of American Type Culture Collection.
11 - PERFORMANCES CHARACTERISTICS

Several experimental works have been published for the evaluation of the specificity and sensitivity of MUCAP Test in identifying Salmonella colonies. The sensitivity values are almost constantly close to 100%,17,19 the specificity values are more variable and sometimes dependent on the isolation and enrichment media combination and vary between 80% and 100%.17,19

The specificity and sensitivity of the detection lactose negative, H₂S positive and MUCAP positive colonies are respectively of 100% and 99.8%.15

Prior to release for sale a representative sample of all lots of MUCAP Test is tested with positive and negative strains.


C₆ esterase negative strains (not fluorescent under Wood’s lamp within 5 minutes): P.mirabilis ATCC 26933, P.vulgaris ATCC 13315, C.freundii ATCC 8090, S.marcescens ATCC 8100, H.alvei CB 910.

12 - LIMITATIONS OF THE METHOD

- Before carrying out the test it is advisable to examine the plates under Wood’s lamp for the presence of natural fluorescence developed by Pseudomonas spp. Strains with natural fluorescence should not be subjected to the MUCAP Test.
- Lactose positive Salmonella strains, with atypical colonies on conventional lactose-containing media such as SS Agar, Hektoen Enteric Agar etc, may not be detected with MUCAP Test; however, these strains can be detected with the reagent on lactose-free media.
- Since most of the false positive strains are oxidase positive,16 the H₂S negative and fluorescence positive colonies can be tested with the oxidase reagent or strips. The combination of MUCAP and oxidase tests raises the specificity of C₆ esterase detection from 94.1% to 98.2%.16
- The reagent does not affect the viability of the flooded colonies.
- Do not take any reading after 5 minutes because the reagent is susceptible to autolysis due to the water nature of the medium and so false positive results might occur.
- The MUCAP Test can be carried-out on the most commonly used culture media (e.g. Mac Conkey Agar, SS Agar , Hektoen Enteric Agar, Brilliant Green Agar, Desoxycholate Agar, XLD Agar etc.). MUCAP Test cannot be performed on colonies cultivated on Bismuth Sulphite Agar.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed for complete identification of the MUCAP positive colonies. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnostic procedures of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative in vitro diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is classified as flammable according to the current European legislation. Consult the Safety Data Sheet before the use.
- Apply good laboratory practice guidelines when performing the test.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as the reagent, the culture media or the microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused reagent and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use this product as a active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the in vitro diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the reagent in the original pack at 2-8°C away from direct light. If properly stored, the reagent may be used up to the expiration date. Do not use the reagent beyond this date. Opened bottle can be used up to the expiration date. Repeated openings of the bottle do not affect the performances and do not cause contamination of the reagent. Do not use the reagent with signs of deterioration (turbidity, precipitate, atypical colour).

191500 MUCAP TEST
SDS rev 4
Regulation (EU) 2020/878
Classification
The product is classified as hazardous

Hazard classification and indication:
- Flammable liquid, category 2
- Aspiration hazard, category 1
- Skin irritation, category 2
- Specific target organ toxicity - single exposure, category 3
- Hazardous to the aquatic environment, acute toxicity, category 1
- Hazardous to the aquatic environment, chronic toxicity, category 1

Hazardous to the aquatic environment, acute toxicity, category 1: H400
- May cause drowsiness or dizziness.

Hazardous to the aquatic environment, chronic toxicity, category 1: H410
- Very toxic to aquatic life with long lasting effects.

Hazardous to the aquatic environment, acute toxicity, category 1: H400
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- Very toxic to aquatic life with long lasting effects.

Hazard labelling
Pictogram

Hazard statements:
- H225 Highly flammable liquid and vapour.
- H304 May be fatal if swallowed and enters airways.
- H315 Causes skin irritation.
- H336 May cause drowsiness or dizziness.
- H410 Very toxic to aquatic life with long lasting effects.

Precautionary statements:
- P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
- P311 Do NOT induce vomiting.
- P280 Wear protective gloves/ protective clothing / eye protection / face protection.
- P301+P310 IF SWALLOWED: Immediately call a POISON CENTER / doctor /...
- P370+P378 In case of fire: use...
- P273 Avoid release to the environment.

Contains: HEPTANE