

## Test Kit for Esculin Hydrolysis

### Intended Use

Test Kit for Esculin Hydrolysis is used for detection of Esculin hydrolysis.

### Summary

Group D Streptococci and Enterococci are able to split esculin. The test is based on detection of esculin hydrolysis and is used for the identification of group D Streptococci.

### Principle

The medium contains esculin, ferric citrate to provide ferric ions, and 1% ox bile to inhibit most other strains of nongroup D Streptococci. Sodium azide is incorporated into the medium to inhibit Gram-negative bacteria. Esculin is hydrolyzed by group D Streptococci to form dextrose and esculetin. Esculetin reacts with the ferric ions contained within the medium, turning the medium dark brown to black. Thus, tolerance to the presence of bile and hydrolysis of esculin provide the means to presumptively identify group D Streptococci. This test can also be used for identification of *Listeria*, since all *Listeria* species hydrolyse esculin.

### Reagent

The Micropress® Test Kit for Esculin Hydrolysis is a reagent set for laboratory use only.

The Micropress® Test Kit for Esculin Hydrolysis comprises of:

1. 10 vials containing 1 mL medium each for esculin hydrolysis.

### Additional Material Required

0.9% Saline, micropipettes, culture media, activated 2% glutaraldehyde solution, sterile test tube, incubator/water bath at 35°C-37°C.

### Directions

#### Preparation of Inoculum

1. Isolate the organism to be identified on Brain Heart Infusion Agar (BHI).
2. Pick up a single well-isolated colony and streak on to BHI agar slant for enrichment and incubate at 35°C-37°C for 18-24 hours.
3. Observe for good growth.
4. Wash the growth with 2-3 mL sterile saline.
5. Match the turbidity of this suspension to McFarland Standard Number 0.5.

#### Inoculation of Vials

1. Bring the medium/vials to the room temperature.
2. Inoculate the vial with 100 µL of the above prepared inoculum.
3. Incubate at 35°C-37°C for 18-24 hours.
4. Observe for growth and colour change.

### Quality Control

**Appearance of the Medium:** Clear, cream to amber coloured medium.

**Cultural Response:** Vials are inoculated with 100 µL culture suspension of following organism, incubated for 18-24 hours at 35°C-37°C.

Organism (ATCC)	Results (Esculin Hydrolysis)
<i>Enterococcus faecalis</i> (29212)	+
<i>Listeria monocytogenes</i> (19112)	+
<i>Streptococcus pyogenes</i> Strain Bruno (19615)	-

**Key:** + = Black colour; - = No change in colour

### Interpretation of Results

1. Development of black colour in the medium indicates a positive test.
2. No change in colour denotes a negative test.

## Remarks

1. The Micropress® Test Kit for Esculin Hydrolysis is an *In vitro* diagnostic kit for laboratory and professional use only. Not for medicinal use.
2. The Micropress® Test Kit for Esculin Hydrolysis cannot be used directly on clinical specimens.
3. Do not use damaged or leaking kits. Avoid contact of reagents with skin and eyes.
4. Clinical samples and microbial cultures should be considered as pathogenic biohazard and handled accordingly. Good laboratory practices and hazard precautions must be observed at all times.
5. Always use pure culture for testing.
6. The test is an aid to identification and is not a confirmatory test. Complete identification should include determination of Gram reaction, morphology, and other biochemical and serological tests.
7. Some strains of *Staphylococcus* and *Aerococcus* can grow in the presence of bile and can hydrolyse esculin.
8. A heavy inoculum on Bile Esculin Azide Media may cause difficulties in interpreting the bile esculin test.
9. Excess inoculum decreases the ability of the ox bile to inhibit the growth of other Gram-positive organisms that may hydrolyse esculin.
10. There are a few Streptococci that do not hydrolyse esculin but will grow in the presence of bile. Growth without blackening of this medium does not constitute a positive test for group D Streptococci.
11. Other test such as Salt Tolerance can be used to distinguish Enterococci.

## Storage and Stability

1. Store the Micropress® Test Kit for Esculin Hydrolysis at 2°C-8°C away from bright light.
2. Stability of the Micropress® Test Kit for Esculin Hydrolysis is as per the expiry date mentioned on the label.

## Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

## Reference

1. C. Chuard and I.B.Reller 1998, Bile Esculin Test for Presumptive Identification of Enterococci and Streptococci. J. Clin. Microbiol.; 36:1135-1136.
2. Practical Medical Microbiology, Mackie and McCartney, 13<sup>th</sup> edition 1989, Edited by J.G.Collee,J.P. Duguid.
3. Data on file: Micropress®, A Division of Tulip Diagnostics (P) Ltd.

## Product Presentation:

Cat. No.	Product Description	Pack Size
203200390001	Ready Prepared Kit	1 Kit (10Tests)

 Temperature Limit	 Manufacturer	<b>REF</b> Catalogue Number	 Date of Manufacture	 Contains sufficient for <n> tests	<b>ESCULIN HYDROLYSIS</b>	<b>TEST</b>
 Use-by Date	 Consult Instructions for use	<b>LOT</b> Batch Code	<b>PS</b> Production Site	 This way up	10 vials containing 1.0 mL medium each for Esculin Hydrolysis	Test for detection of Esculin Hydrolysis by Group D Streptococci

Revision: 0825/VER-03

## Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.