



## 1% FERRIC AMMONIUM CITRATE (ESCULIN HYDROLYSIS REAGENT)

### INTENDED USE

Remel 1% Ferric Ammonium Citrate reagent is recommended for use in qualitative procedures for the detection of esculin hydrolysis in anaerobes.

### SUMMARY AND EXPLANATION

Certain anaerobic gram-negative bacilli (i.e., *Bacteroides fragilis*, *Fusobacterium mortiferum*) and several species of the genus *Clostridium* are capable of hydrolyzing esculin. The esculin hydrolysis test can be used to differentiate esculin-positive strains from esculin-negative species in the same genus.<sup>1-3</sup>

### PRINCIPLE

When esculin is hydrolyzed by bacteria in the presence of 1% Ferric Ammonium Citrate, black ferric salts precipitate out into the medium.

### REAGENTS (CLASSICAL FORMULA)\*

Ferric Ammonium Citrate (CAS 1185-57-5) ..... 10.0 g  
Demineralized Water (CAS 7732-18-5) ..... 1000.0 ml

\*Adjusted as required to meet performance standards.

### PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information on reagent chemicals.

### STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Protect product from light.

### PRODUCT DETERIORATION

This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

### SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.<sup>4</sup>

### MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swab, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Ultraviolet light, (7) Esculin Broth w/ 0.07% Agar (R060872).

### PROCEDURE

1. Inoculate Esculin Broth w/ 0.07% Agar from a pure, 18-24 hour culture of the test isolate.
2. Incubate anaerobically at 35-37°C for 24-48 hours.
3. Add 3-5 drops of 1% Ferric Ammonium Citrate.
4. Observe for a black-brown color development.

5. Alternatively, the tube may be examined for fluorescence under a longwave ultraviolet light (365 nm).<sup>2</sup> Loss of fluorescence indicates a positive reaction. Blue fluorescence indicates a negative reaction.

**Note:** If the colony is an H<sub>2</sub>S-producer, the broth must be examined for fluorescence as H<sub>2</sub>S obscures esculin hydrolysis.<sup>2</sup>

### INTERPRETATION

- Positive Test - Black-brown color development or loss of fluorescence under long wave ultraviolet light
- Negative Test - No color development or blue fluorescence under long wave ultraviolet light

### QUALITY CONTROL

All lot numbers of 1% Ferric Ammonium Citrate have been tested using the following quality control organisms and have been found to be acceptable. Testing of a positive and negative control should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

### CONTROL

*Bacteroides fragilis*  
ATCC® 25285

*Prevotella melaninogenica*  
ATCC® 25845

### INCUBATION

Anaerobic, up to  
48h @ 35-37°C

Anaerobic, up to  
48h @ 35-37°C

### RESULTS

Positive

Negative




### BIBLIOGRAPHY

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2. Dowell, Jr., V.R., G.L. Lombard, F.S. Thompson, and A.Y. Armfield. 1987. Media for Isolation, Characterization, and Identification of Obligately Anaerobic Bacteria. U.S. Dept. of H.H.S. and CDC, Atlanta, GA.
3. Dowell, Jr., V.R. and T.M. Hawkins. 1990. Laboratory Methods in Anaerobic Bacteriology. U.S. Dept. of H.H.S. and CDC, Atlanta, GA.
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5. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey and Scott's Diagnostic Microbiology. 11<sup>th</sup> ed. Mosby, St. Louis, MO.
6. Holdeman, L.V. and W.E.C. Moore. 1977. Anaerobe Laboratory Manual. 4<sup>th</sup> ed. Virginia Polytechnic Institute, Blacksburg, VA.

### PACKAGING

REF R21215, 1% Ferric Ammonium Citrate .....25 ml/Btl

### Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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CAS (Chemical Abstracts Service Registry No.)

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