

## Tween 80 Hydrolysis

### Intended Use

Tween 80 Hydrolysis is a biochemical test for differentiation of saprophytic species of Mycobacteria.

### Summary

Many a times, *Mycobacterium* Other Than Tuberculosis (MOTT) may be the cause of disease in humans and other animals. Various biochemical and biological criteria have been used to identify and differentiate *M. tuberculosis* from MOTT. The identification of Mycobacteria to the species level is important because of the clinical significance; some species are pathogenic while others are not. Knowledge of species is also critical in order to provide adequate patient management because specific antimycobacterial drugs are required against different pathogenic Mycobacteria species. Biochemical tests should be undertaken for differentiation of clinically significant pathogenic scotochromogens, photochromogens and non-chromogens from the commonly saprophytic representations of these groups.

The Tween 80 Hydrolysis test is used for differentiation of pathogenic species of scotochromogens, photochromogens and non-chromogens from the non-pathogenic members of these groups. The test can also be used for the confirmation of *M. kansasii* (which produces a positive test in 3 to 6 days), and can also be used to distinguish between two scotochromogens *M. gordonae* (positive) and *M. scrofulaceum* (negative).

### Principle

The commonly non-pathogenic, slow growing scotochromogens and non-chromogens produce a lipase that is able to hydrolyse Tween 80 into oleic acid and sorbitol, while the saprophytic species of these groups are unable to hydrolyse Tween 80. Intact Tween 80 binds neutral red indicator to give an amber coloured complex. When Tween 80 is hydrolysed, it can no longer bind with the neutral red indicator. The neutral red indicator assumes its normal configuration at pH 7, which is pink-red in colour.

### Reagent

Microexpress® Tween 80 Hydrolysis kit is a reagent for laboratory use only.

Tween 80 Hydrolysis kit; is a biochemical test for differentiation of saprophytic species of photochromogens, non-chromogens and scotochromogens. It comprises of:

1. Concentrated mixed Tween 80 Hydrolysis reagent.

The Tween Hydrolysis substrate reagent used in the test can be prepared from concentrated mixed Tween 80 Hydrolysis reagent of the Tween 80 Hydrolysis kit.

### Additional Material Required

Biosafety hood, incubator, 3 mm sterile plating loops, activated 2% glutaraldehyde solution, 10 µL-100 µL, 0.1-1 mL variable pipette, screw cap test tubes (16 X 125 mm) with 2 mL sterile distilled water, test tube stand, sterile distilled water or sterile isotonic saline.

### Directions

1. Label the required number of sterile screw caps tube(s) containing Tween 80 Hydrolysis substrate reagent, to correspond with the culture identity, to be tested and a blank without any culture.
2. Using a 3 mm sterile loop, draw a loopful of the culture to be tested and inoculate the corresponding labelled Tween 80 Hydrolysis substrate reagent following aseptic conditions. A fresh disposable loop should be used for each inoculation.
3. Close the screw cap of the inoculated Tween 80 Hydrolysis substrate reagent and incubate at 35°C-37°C.
4. Observe for change in colour of the Tween 80 Hydrolysis substrate reagent from amber to pink-red at 24 hours, 5 days and 10-12 days. Always compare with the blank to see change in colour.
5. Do not shake the Tween 80 Hydrolysis substrate reagent tubes while reading.

### Preparation of Tween 80 Hydrolysis Substrate Reagent

1. Bring the concentrated mixed Tween 80 Hydrolysis reagent to room temperature prior to use.
2. Use the required number of sterile screw-capped tubes/bottles of size 16 x 125 mm containing 2 mL of sterile distilled water.
3. Aseptically pipette out in each test tube 100 µL of the concentrated mixed Tween 80 Hydrolysis reagent.

4. Slightly warm (50°C) this solution to enable complete dissolution of Tween 80 by gentle swirling.
5. Close the screw cap tightly.
6. The amber coloured solution thus obtained is Tween 80 Hydrolysis substrate reagent.
7. Allow the reagent to cool prior to testing.

### Quality Control

**Appearance:** Red viscous liquid.

**Cultural Response:** Cultural response observed after an incubation of 10-12 days at 35°C-37°C.

### Organisms (ATCC)

*Mycobacterium tuberculosis* H37Rv strain

*Staphylococcus aureus* subsp. *aureus* (25923)

### Results

Pink colour formation in 10-12 days

No colour change till 12 days

### Interpretation of Results

1. Change in colour, of the Tween 80 Hydrolysis substrate reagent in the screw cap tube and not the cells, from amber to pink-red is a positive test result.
2. Results are to be read at 24 hours, recorded and positive tubes discarded.
3. Negative results are to be further incubated and read after 5 days, recorded and positive tubes discarded.
4. Tubes, which are negative after 5 days, are to be further incubated for 10-12 days.
5. Record final results after 10-12 days and then discard all tubes.

### Performance Characteristics

The performance standards of the above product conform to the NCCLS standards of quality assurance for commercially prepared microbiology products.

### Remarks

1. During incubation, the tubes should be protected from light to minimize spontaneous decolorization of the neutral red indicator.
2. The test procedure must be carried out under a biosafety hood.
3. Discard the screw caps tubes following good laboratory practices, once the results are read and recorded.
4. Treat the specimens, equipment's and used slants by immersing in 2% activated glutaraldehyde for at least two hours before incineration and disposal.
5. Good laboratory practices and hazard precautions must be observed as all times.
6. All culture growth should be characterized based on morphology, AFB stain and biochemical tests.

### Storage and Stability

1. Store the Tween 80 Hydrolysis kit at 2°C-8°C, away from light.
2. Stability of the Tween 80 Hydrolysis kit 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. Diagnostic Key to Mycobacteria Encountered in Clinical Laboratories; Lawrence G. Wayne and Joseph R. Dubek; Applied Microbiology, No.:6, June 1968, p 925-931.
2. Rapid Tween-80 Hydrolysis Test for Mycobacteria; F.R. Cox. *et al.*; Journal of Clinical Microbiology; Vol.: 7, No.: 1; Jan 1978, p. 104 105.
3. Preparation of a Stable Mycobacterial Tween Hydrolysis Test Substrate; J.O. Kilburn *et al.*, Applied Microbiology; Vol.: 26, No.: 5, 1973, p: 826.
4. Clinical Diagnosis and Management by Laboratory Methods, Todd, Sanford & Davidsohn, 17<sup>th</sup> Edition, 1998, edited by John Bernard Henry.
5. Tuberculosis; A Clinical Handbook, 1<sup>st</sup> Edition 1995, edited by L. I. Lutwick.
6. Mycobacteriology; Laboratory methods for clinical and public health; U.S. department of health, education and welfare, Public Health Service Publication No. 1547.

7. Procedures for the isolation and identification of Mycobacteria; U.S. department of health, Education, and Welfare, C.D.C, 1975 Edition.
8. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

**Product Presentation:**

| <b>Cat. No.</b> | <b>Product Description</b> | <b>Pack Size</b> |
|-----------------|----------------------------|------------------|
| 203200450100    | Ready Prepared Kit         | 100 Tests        |

**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.

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