thermoscientific

Instructions for Use

Thermo Scientific™ Sensititre™
MIC Susceptibility Plates
for Mycobacterium tuberculosis

011 – MYCOTB –CID10470
Revision Date: October, 2019

MIC Susceptibility Plates
For Mycobacterium tuberculosis

Sensititre 72 hour

For *Research Use Only*

For full plate information, including plate layout, QC information, Interpretative criteria, performance data and references please refer to www.trekds.com/techinfo. The plate code and batch number will be required.

INTENDED USE
The MYCOTB plate is for susceptibility testing of Mycobacterium tuberculosis. The procedure outlined below is for visual reading of growth. Broth microdilution methodology has been used to study drug activity (references 8 and 9) and performance has been compared to the standard agar proportion method (reference 10).

Thermo Scientific manufactured products have only been validated with Sensititre broth.

SUMMARY AND PRINCIPLES OF USE
The MYCOTB plate consists of a 96 well microtiter plate containing twelve antimicrobial agents at appropriate dilutions and two positive growth controls. It is recommended that the test is set up using saline-tween with glass beads and Middlebrook 7H9 broth with OADC. Results can be read manually by visually reading growth.

PRECAUTIONS
This product is for research use only and should be used by properly trained personnel. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, media, and test plates after use. Directions should be read and followed carefully.

NOTE: The laboratory should have established biosafety guidelines for handling mycobacteria.

STORAGE AND SHELF LIFE
The plates should be stored at room temperature (15-25°C) away from direct sunlight and direct heat. Each plate is packaged in foil with a silica gel desiccant. Do not use the plate or broth if past its expiration date, or the desiccant color is not blue or orange or the foil pouch is damaged.

Inoculate plate within 5 hours of removal from pouch

SPECIMEN COLLECTION AND PREPARATION
Specimens should be collected, transported, stored and then plated on to primary isolation medium according to established microbiological laboratory practice to give isolated colonies.

PROCEDURE
Materials included:

10 Sensititre plates without substrate in wells.
10 Adhesive seals

Materials not included [Inc Product Code]:

Broth:
Sensititre™ Vizion/SWIN system [V2020]
Sensititre Middlebrook 7H9 w/OADC
Sensititre Saline Tween with glass beads [T3490]

Turbidity:
Sensititre™ Nephelometer [V3011]
0.5 McFarland turbidity standard

Plate inoculation:
Doseheads for Sensititre AIM for plate inoculation
Sensititre Automated Inoculation Delivery System **(AIM)** [V3020] / Sensititre Autoinoculator*

Plate Reading:
Thermo Scientific™ Sensititre™ Manual Viewer [V4007]

Other Supplies:

Quality control strains, *M.tuberculosis* ATCC® 27294™
50 or 100µl pipettor and disposable tips
Middlebrook 7H10 agar plates
Blood agar plates
Incubator 35-37°C, non-CO_2
Vortex mixer
Seed trough or empty Petri dish

SELECTION OF SUSCEPTIBILITY TEST BROTH

All Sensititre approved broths are performance tested for use in Sensititre susceptibility products. It is advisable to se Sensititre approved Saline Tween with glass beads and 7H9 broth supplemented with OADC

INOCULATION PROCEDURE

Allow all broths to come up to room temperature before use.

1. Scrape colonies from agar plate and emulsify in a tube containing saline, 0.2% tween and glass beads.
NOTE: Cultures used for testing should not be older than 5 weeks.
2. Vortex for a minimum of 30 seconds.
3. Adjust turbidity to a 0.5 McFarland Standard visually or using the Sensititre Nephelometer. If utilizing the Sensititre Nephelometer, instrument must be calibrated with the 0.5 Polymer McFarland StandardAllow to settle for 15 minutes.
4. Transfer 100 µl of the suspension into a tube of 7H9 broth supplemented with OADC to give an inoculum of $5x10^5$ cfu/ml (Range $5x10^4$ to $5x10^5$ cfu/ml). Vortex for 30 seconds.
5. Steps 1 to 4 must be completes within 30 minutes.
6. Inoculate the plate using an appropriate multi-channel pipette. Transfer 100µl into each well. Dose the panel with the Sensititre label facing the user. Pipettes should be periodically serviced and checked for calibration. If using a Sensititre AutoInoculator /AIM utilize a dosehead during inocula process.

Remove the test tube/dosehead combination from the AutoInoculator / AIM within 30 seconds of dosing a plate and store inverted in a rack or discard.

Inoculate broth into the Sensititre plate within 30 minutes.

7. A periodic check of the inoculum density by colony count from the positive well should be done. (See Appendix 1). Isolates should have an inoculum of $5x10^5$ cfu/ml (range $5x10^4 – 5x10^5$).

8. Cover all wells with the adhesive seal. Press all wells firmly to assure adequate sealing. Avoid creases as these can lead to skips. Wipe the sealed plate with a disinfectant before transferring to the incubator. Recommended disinfectants include phenol based compounds such as Amphyl.

9. Incubate plates aerobically at 35-37°C for 10 days. Check for growth at 10 days. If growth is poor after 10 days, reincubate plate up to an additional 11 days. Plates can be stacked up to two-high during incubation.

PURITY CHECK

To verify the culture is a single organism, a purity check is required. From the Middlebrook 7H9- OADC suspension, streak 50µl onto a blood agar (BAP) and Middlebrook 7H10 plate. Incubate the plates at 35-37°C for 48-72 h for the BAP, and up to 8 weeks for the 7H10 plates

READING TEST RESULTS

Results can be read using the Sensititre manual viewer or the Vizion. See Vizion User Manual. It is not necessary to remove the adhesive seal. Place the plate with the label facing the user. Growth appears as turbidity or as a deposit of cells at the bottom of a well. The MIC is recorded as the lowest concentration of antimicrobic that inhibits visible growth. Reading faint growth on Vizion can be improved by use of bright indirect lighting against a dark background.

The positive growth control wells should be read first. If any show no growth, results are invalid.

Mycobacteria end points can be difficult to interpret. CLSI M24 provides reading guidelines and illustrations of various growth patterns. Negative wells can show a slight precipitate related to the inoculum. Reading QC strains with known MICs should be used for training

Growth can range from a few colonies with no turbidity to heavy growth comparable to positive growth control. The MIC is the lowest concentration that completely inhibits growth except for sulphonamides, where the MIC is read as the lowest concentration that inhibits 80% growth compared to the positive control.

The following points should be noted:

a. Contamination

Contamination may result in growth in a well bordered by wells showing no growth. Such a single well contamination can be ignored, but if multiple well contaminants are suspected, the test should be repeated.

b. Skips

Occasionally a “skip” may be seen - a well showing no growth bordered by wells showing growth. There are variety of explanations including contamination, mutation, creased seal and misaligned dosing. A single skip can be ignored. However, in order to ensure effective antimicrobic therapy NEVER read the skipped well as the MIC; always read the lowest

well concentration above which there is consistently no growth.

c. Mixed Cultures

Except as referred to in (a) above, if two end points are seen as a distinct "button" of cells followed by several wells of diffuse growth with the "button" no longer visible (or seen as smaller buttons), there may be a mixed bacterial population. Purity should be checked by sub-culturing growth onto suitable agar. Test results are invalid if a mixed culture is detected.

INTERPRETATION OF RESULTS

Interpretative guidelines for broth microdilution testing have not been developed at this time.

QUALITY CONTROL

Frequency of quality control testing should be according to local guidelines. Inocula should be cultured on a suitable medium to check for purity and/or colony morphology composition. Test results are invalid if a mixed culture is detected.

All Sensititre plates include positive control wells. Tests are invalid unless there is distinct growth in all positive control wells

A number of factors influence MIC's including organism state, inoculum density, temperature and broth. In practice, replicate MIC's form a normal distribution with the majority of results lying within one dilution of the modal value. The test procedure can be considered satisfactory if control organism MIC's are within range. Results should **not** be reported if QC results are not in range.

Table 1 lists tentative QC ranges using *Mycobacterium tuberculosis* strains.

The procedure for inoculating and reading test results for this strain is the same as described in previous sections. If CLSI M100 (non Myco) QC strains are used to test the performance, the procedure is the same except that the bacterial suspension is prepared in sterile water. 50µL of the suspension is added to 10mL of Sensititre Mueller Hinton broth. Plates are read after 16-20 hours incubation at 34°C-36°C.

Table 1:

Tentative Quality Control Ranges[1] for *M. tuberculosis* QC strains

Drug	*M. tuberculosis* ATCC 27294
Amikacin	0.25-2
Cycloserine	4.0-16.0
Ethionamide	0.6 - 5
Ethambutol	≤2
Isoniazid	≤0.5
Kanamycin	0.6-5
Moxifloxacin	≤ 0.5
Ofloxacin	0.5 - 2
PAS	≤0.5
Rifampin	≤0.5
Rifabutin (Ansamycin)	≤ 0.12
Streptomycin	0.5-2

Note: [1] Ranges based on in-house testing

Contact Sensititre Distributor or Thermo Fisher Scientific Technical Services for assistance in the event that quality control discrepancies cannot be resolved. See final page for contact information.

LIMITATIONS

1. Performance of the procedure has not been established at this point.
2. Results are invalid if the 7H9 broth is not supplemented with OADC.
3. Organisms other than *M. tuberculosis* should not be tested using this MIC plate.

APPENDIX 1: Colony Count Procedure

1. Immediately following inoculation plate, using a 1µl loop, sample from the positive growth control well and inoculate onto Middlebrook 7H10 agar plate.
2. Take another loop (1µl) and sample from the same growth well and mix with 50µl sterile deionized water. Inoculate 1µl of this dilution onto a Middlebrook 7H10 agar plate to obtain countable colonies.
3. Incubate both plates at 35–37 °C for up to 14 days under appropriate conditions.
4. Read as follows:

Number of colonies

Colony Count	0.001 plate	0.001 of 1/50 dilution
$<5 \times 10^4$	<50	0
5×10^4	50-100	0-2
$1 \times 10^5 - 5 \times 10^5$	>100	$\underline{<}$ 10
$>5 \times 10^5$	>100	>10

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