



## Wayne Sulphatase Agar Base

M1059

Wayne Sulphatase Agar Base is used for biochemical differentiation of *Mycobacteria* on the basis of their ability to produce arylsulphatase.

### Composition\*\*

Ingredients	Gms / Litre
Casein enzymic hydrolysate	0.500
L-Asparagine	1.000
Monopotassium phosphate	1.000
Disodium phosphate	2.500
Ferric ammonium citrate	0.050
Magnesium sulphate	0.010
Calcium chloride	0.0005
Zinc sulphate	0.0001
Copper sulphate	0.0001
Tripotassium phenolphthalein sulphate	0.650
Agar	15.000
Final pH ( at 25°C)	7.0±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 20.71 grams in 1000 ml distilled water containing 10 ml glycerol. Heat to boiling to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubed media to cool in an upright position.

### Principle And Interpretation

*Mycobacteria* have traditionally been treated as a separate group of bacteria in most clinical laboratories because of certain distinguishing characteristic. First, most strains are slow growing, having prolonged doubling time, ranging from 2-22 hours. This requires an ideal culture environment to be maintained for prolonged periods and the more rapidly growing contaminating bacterial species to be eliminated from the specimens. Determination of the enzyme arylsulphatase activity in *Mycobacteria* is helpful in identifying certain species, notably in differentiating members of the rapidly growing *Mycobacteria fortuitum* from group III non-photochromogenic *Mycobacteria*. Some of the slower-growing species do not produce sufficient enzyme to give a consistently positive reaction.

Wayne Sulphatase Agar was developed by Wayne (to enable recognition of *M.fortuitum*) using a 3-day phenolphthalein sulphatase test. Rapid growing and slow growing species of *Mycobacterium* can be differentiated, based on the 3 days test or 2 weeks test respectively. Some species of *Mycobacteria* produce arylsulphatase, an enzyme that attacks the substrate component viz. tripotassium phenolphthalein sulphate, with the resultant release of free phenolphthalein as indicated by a colour change (red) in the medium after addition of sodium bicarbonate reagent. After incubation of 3-14 days, add 0.5 to 1.0 ml of 2N Na<sub>2</sub>CO<sub>3</sub> to each tube and observe the colour change within 30 minutes.

### Quality Control

#### Appearance

Cream to yellow homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel.

#### Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in tubes as butts.

#### Reaction

Reaction of 2.07% w/v aqueous solution at 25°C. pH : 7.0±0.2

**pH**

6.80-7.20

**Cultural Response**

M1059: Cultural characteristics observed after an incubation at 35- 37°C for 3 days (Mycobacterium tuberculosis incubated for 2 weeks).

**Organism****Growth**

*M. tuberculosis H37 RV*  
25177

light to heavy  
growth with  
negative  
reaction.

*Mycobacterium fortuitum*  
ATCC 6841

moderate to  
heavy growth.  
Light to dark  
pink (positive)  
reaction.

**Storage and Shelf Life**

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

**Reference**

1.Wayne L. G., 1961, Am. J. Clin. Pathol. 36:185. 2.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria Vol. 1, Williams & Wilkins, Baltimore.

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