# Wayne Sulphatase Agar Base

**M1059**

## Intended Use:

Recommended for biochemical differentiation of *Mycobacteria* on the basis of their ability to produce aryl sulphatase.

## Composition**

| Ingredients | g / L |
|---|---|
| Tryptone | 0.500 |
| L-Asparagine | 1.000 |
| Potassium dihydrogen phosphate | 1.000 |
| Disodium hydrogen 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 purified/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 tubes 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 (1).

## Type of specimen

Clinical samples : Sputum

## Specimen Collection and Handling

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 $Na_2CO_3$ to each tube and observe the colour change within 30 minutes. After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations

1. Well isolated colonies must be used for erroreous results.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## 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**

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 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

## 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

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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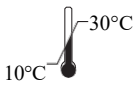
IVD **_In vitro_ diagnostic medical device**




**Storage temperature**

EC REP

CEpartner4U, Esdoornlaan 13,
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CE **CE Marking**

**Do not use if package is damaged**

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