HARMONIZED HIMEDIA®

Technical Data

# Dilute Sautan's Medium (Twin Pack)

**MM1276**

## Intended Use:

Recommended for cultivation and enumeration of Mycobacteria, in accordance with IP.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Part A | - |
| Ferric ammonium citrate ( brown) | 0.017 |
| L-Asparagine | 1.330 |
| Magnesium sulphate heptahydrate | 0.167 |
| Dipotassium hydrogen phosphate | 0.178 |
| Sodium dihydrogen phosphate | 0.057 |
| Sodium chloride | 0.036 |
| Polysorbate 80 | 0.833 |
| Part B | - |
| Citric acid | 0.667 |
| Final pH ( after sterilization) | 7.2±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 2.52 grams (the equivalent weight of dehydrated medium per litre) of Part A in 500 ml purified/distilled water containing 20 ml glycerol. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 20 minutes. Suspend 0.667 grams of Part B in 500 ml distilled water. Sterilize by autoclaving at 121°C for 20 minutes. After autoclaving mix Part A and Part B solution aseptically. Cool to 45-50°C. Mix well and dispense into sterile tubes or flasks as desired.

*Note: Directions specified are as per the concurrent edition of pharmacopoeia in force. Specified expiry period corresponds to this. User must ensure its compatibility with the latest edition.*

## Principle And Interpretation

Dilute Sauton's Medium is used for determining the number of colony forming units (CFU) in vaccines of known potency as per I.P. (1, 2). Salts like ferric ammonium citrate and magnesium sulphate provide inorganic ions and nitrogen sources essential for the growth of Mycobacteria. Asparagine is added to promote the initiation of growth and increase the growth rate. Glycerol in the medium serves as a carbon source.

## Type of specimen

Pharmaceutical sample

## Specimen Collection and Handling:

As per the I.P. method, contents of the freeze-dried vaccine for human use are reconstituted with the diluent stated on the vial. Contents of 5 such containers are pooled and three dilutions of the pooled vaccine are prepared using Dilute Sauton's Medium so as to obtain an optimum of 100, 40 and 20 colonies from an inoculum of 0.2 ml. An inoculum of 0.2 ml from each dilution is further inoculated onto L.J. Medium (MM162) slant surface and incubated at 35-37°C for 4-5 weeks. The vaccine passes the test if 0.1 ml of the reconstituted vaccine contains between 1 $x10^5$ and 33 x $10^5$ colony forming units. After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions

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 specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations:

1. Further biochemical and serological tests must be carried out for further identification.

## 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**

Part A : Cream to off-white homogeneous free flowing powder

Part B : White to off-white homogeneous free flowing powder

**Colour and Clarity of prepared medium**

Light yellow coloured, clear to slightly opalescent solution of complete medium

**pH**

7.00-7.40

**Cultural Response**

Cultural response was observed on L.J.Medium using dilute Sautans Medium as a diluent after an incubation at 35-37°C for 4-5 weeks.

| Organism | Inoculum (CFU) | Growth (on L.J.Medium) |
|---|---|---|
| *Mycobacterium smegmatis* ATCC 14468 | 20-100 | Good *(Clearly visible growth) |
| *M. tuberculosis* H37RV(25618) | 20-100 | Good *(Clearly visible growth) |

## Storage and Shelf Life

Store below 10-30°C in a tightly closed container and the prepared medium at 15-25°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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

## Reference

1. Atlas R. M., 1993, Handbook of Microbiological Media, Parks L (Ed.) CRC Press, Inc.

2. Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.

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

4. 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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