Iron Oxidizing Medium (Twin Pack)

M615

Intended Use:

Recommended for for the isolation, cultivation and enrichment of *Thiobacillus ferroxidans.*

Composition**

Ingredients	Gms / Litre
Part A	-
Ammonium sulphate	3.000
Potassium chloride	0.100
Dipotassium hydrogen phosphate	0.500
Magnesium sulphate heptahydrate	0.500
Calcium nitrate	0.010
Part B	-
Ferrous sulphate heptahydrate	44.220
Final pH (at 25°C)	3.3±0.3

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 3.85 grams (the equivalent weight of dehydrated medium per litre) of Part A in 700 ml purified / distilled water containing 1 ml of 10N sulphuric acid. Heat to boiling to dissolve the medium completely. Suspend 44.22 grams of Part B separately in 300 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Sterilize Part A and Part B separately by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool each solution to 25°C. Aseptically add 300 ml of sterilized Part B to 700 ml of Part A. Mix thoroughly. Aseptically distribute into sterile tubes or flasks.

Principle And Interpretation

Thiobacillus ferrooxidans is recognized as being responsible for the oxidation of iron and inorganic sulfur compounds in areas such a mine tailings and coal deposits where these compounds are abundant (4,7). The main importance of *T. ferrooxidans* has been in acid mine drainage. *T. ferrooxidans* is generally assumed to be obligately aerobic, but under anaerobic conditions, *T. ferrooxidans* can be grown on elemental sulfur using ferric iron as an electron acceptor.

These results indicate that *T. ferrooxidans* can be considered as facultative anaerobe playing an important role in the iron and sulfur cycles in acidic environments. The ability of this organism to grow in oxygen-deficient environments may have important implications in bioleaching processes where anaerobic conditions may often exist (5). Iron Oxidizing Medium (*Thiobacillus ferroxidans*) is formulated in accordance with APHA (1) and is used for isolation, cultivation and enrichment of *T. ferroxidans.*

Magnesium sulphate, ammonium sulphate, potassium chloride and calcium nitrate are sources of ions that stimulate metabolism. Dipotassium phosphate buffers the medium. The medium has a precipitate, is opalescent and green in colour.

T. ferroxidans utilizes ferrous sulphate as energy source. Some oxidation of iron occurs during sterilization. *T. ferroxidans* can be enumerated by MPN technique (6). Growth of the organism is manifested by a decrease in pH and an increase in concentration of oxidized iron. With the use of uninoculated controls, an increase of deep orange brown colour can be seen in positive enrichment tubes or flasks as compared to negative ones.

Type of specimen

Water samples

Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(1) 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. The organisms are highly / strictly aerobic, so the tubes should be shaken every day during incubation.

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 of Part A

White to cream homogeneous free flowing powder.

Appearance of Part B

Greenish yellow to dark green homogeneous hygroscopic powder

Colour and Clarity of Prepared medium

Brownish yellow clear to slightly opalescent with precipitate.

Reaction

Reaction of (0.39 gm Part A in70 ml distilled water containing 1 ml of 10N sulphuric acid)+ 4.42 gm of Part B in 30 ml distilled water at 25°C. pH : 3.3±0.3

pH

3.00-3.60

Cultural Response

Cultural characteristics observed after an incubation at 30°C upto 5 days.

Organism	Growth
Thiobacillus ferrooxidans ATCC 23270	luxuriant

Storage and Shelf Life

Store between 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. 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 (2,3).

Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
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.
4. McGoran C. J .M., Duncan D. W. and Walden C. C., 1969, Can. J.Microbiol., 15:135.
5. Pronk T. T., de Bruyn J. C., Bos P. and Kuenen J. G., 1994Appl. Environ. Microbiol., 58. 2227-2230.
6. Silverman M. P. and Lundgren D. C., 1959, J. Bacteriol 77:642.
7. Unz R. F. and Lundgren D. G., 1961, Soil Sci., 92:302.

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