TM 133 - IRON OXIDIZING MEDIUM (DOUBLE PACK)

INTENDED USE

For isolation, cultivation and enrichment of *Thiobacillus ferrooxidans.*

PRODUCT SUMMARY AND EXPLANATION

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. 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. Iron Oxidizing Medium (*Thiobacillus ferroxidans*) is formulated in accordance with APHA and is used for isolation, cultivation and enrichment of *T. ferroxidans.*

COMPOSITION

Ingredients	Gms / Ltr
Part I	-
Ammonium sulphate	3.000
Potassium chloride	0.100
Dipotassium hydrogen phosphate	0.500
Magnesium sulphate heptahydrate	0.500
Calcium nitrate	0.010
Part II	-
Ferrous sulphate heptahydrate	44.220

PRINCIPLE

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. 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.

INSTRUCTION FOR USE

- Dissolve 4.11 grams of Part I in 1000 ml purified/distilled water containing 1 ml of 10N sulphuric acid.
- Heat to boiling to dissolve the medium completely.
- Dissolve 44.22 grams of Part II 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 psi 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.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Part I: White to cream homogeneous free flowing powder.
Part II: Greenish yellow to dark green homogeneous hygroscopic powder.
Appearance of prepared medium : Brownish yellow clear to slightly opalescent with precipitate.
pH (at 25°C) : 3.3±0.3

INTERPRETATION

Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Incubation Temperature	Incubation Period
Thiobacillus ferrooxidans	23270	50-100	luxuriant	30°C	upto 5 days

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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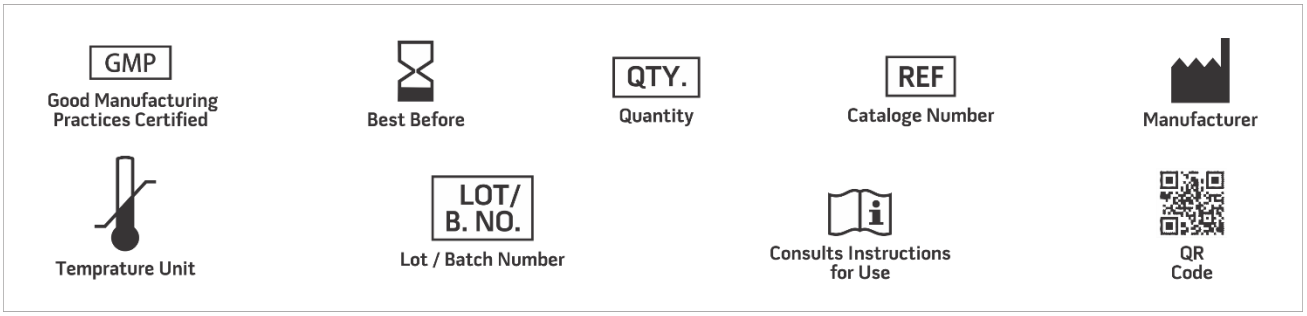

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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