Product Name: 9K Broth

Medium for the enumeration and maintenance the *Thiobacillus spp.*.

FORMULA (G/L)

Ammonium Sulfate	3.0
Potassium Chloride	0.1
Potassium Phosphate Dibasic	0.5
Magnesium Sulfate Heptahydrate	
Calcium Nitrate	
Final pH = 7.3 ± 0.2 at 25° C.	

DIRECTIONS

Suspend 4.11 G of powder in 700 mL of distilled or deionized water. Heat until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45-50°C. Aseptically add 2 vials of 9K Broth Iron Solution (MB-K0780). Mix well. Dispense into final containers. If necessary, before pouring adjust the pH to 2.5 \pm 0.2 by the dropwise addition of H_2SO_4 .

9K Broth Iron Solution

1 Vial contents (Each vial is sufficient for 500 mL of medium)
Ferrous Sulfate Heptahydrate......22.0 G
(Dissolved in distilled water previously acidified to pH 2.5 with H₂SO₄)

EXPLANATION

9K Broth is used for the enumeration and maintenance the *Thiobacillus spp.*.

TECHNIC

Incubate at 28 \pm 2°C for 3 - 4 days in shake culture.

USER QUALITY CONTROL

Dehydrated medium

Appearance: free-flowing, homogeneous.

Color: white.
Prepared medium

Appearance: slightly opalescent.

Color: colorless.

Incubation conditions: $28 \pm 2 \,^{\circ}\text{C} / 3 - 4 \,\text{days}$.

Microorganism	ATCC	Growth
Thiobacillus ferrooxidans	19859	good



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LIMITATIONS OF THE PROCEDURE

9K Broth could be stored for at least two weeks at 2-6°C without noticeable oxidation. The medium exhibited a precipitate (probably ferrous and ferric phosphates), was opalescent and green, had a pH of 3.0 to 3.6, and contained 9000 ppm ferrous iron. The culture has been maintained for more than a year by transferring twice a week into 100 mL of modified medium dispensed in 250 mL Erlenmeyer flasks which were incubated at 28 °C on a reciprocal shaker.

STORAGE

The powder is very hygroscopic: store the powder at room temperature, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident. Store prepared medium at 2-6°C.

REFERENCES

1. Melvin P.S., and Donald G. L., (1958).

PACKAGING

Cat. No : MB-K0729 9K Broth 500 G

