

Technical Data Sheet

Product Name	ATCC 2039 Broth (Twin Pack)
Catalogue Number	AS-1441
Reference	ATCC Medium 2039 — Official ATCC Specification (Acidithiobacillus ferrooxidans Medium)
Pack Format	Twin Pack: Solution A (Salt Base Powder incl. Wolfe's Mineral) + Solution B (FeSO ₄ ·7H ₂ O Powder)
Target Organism	Acidithiobacillus ferrooxidans ATCC 23270; acidophilic iron-oxidising bacteria
HS Code	3821.00.00

Product Description

ATCC 2039 Broth Twin Pack (AS-1441) is a two-component dehydrated culture medium formulated exactly to the official ATCC Medium 2039 specification for the cultivation of Acidithiobacillus ferrooxidans ATCC 23270 and related acidophilic chemolithotrophs. Solution A contains the inorganic salt base with Wolfe's Mineral Solution incorporated. Solution B contains ferrous sulphate heptahydrate, which must be prepared and filter-sterilised separately to prevent iron oxidation. The formulation follows exactly the ATCC reference: Solution A (salts + 5 mL Wolfe's Mineral per litre) + Solution B (FeSO₄·7H₂O 20g per 200 mL), combined aseptically at a ratio of 800:200 mL.

Solution A — Complete Formula (per litre of final medium)

Ingredient	g / L or mL/L	Function
Diammonium Sulphate ((NH ₄) ₂ SO ₄)	0.800 g	Sole nitrogen source for autotrophic growth
Magnesium Sulphate Heptahydrate (MgSO ₄ ·7H ₂ O)	2.000 g	Essential macro-mineral; enzyme cofactor
Dipotassium Hydrogen Phosphate (K ₂ HPO ₄)	0.400 g	Phosphorus source; pH buffer
Wolfe's Mineral Solution (see below)	5.0 mL	Trace mineral supplement — see Wolfe's formula
DI / Purified Water	800.0 mL	Solvent for Solution A

Wolfe's Mineral Solution — Complete Formula (per litre of Wolfe's solution)

Ingredient	g / L	Function
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Nitrilotriacetic acid	1.500	Chelating agent — keeps trace metals in solution; dissolve first, adjust pH to 6.5 with KOH
Magnesium Sulphate Heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	3.000	Macro-mineral supplement
Manganese Sulphate Monohydrate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$)	0.500	Trace mineral; enzyme cofactor
Sodium Chloride (NaCl)	1.000	Ionic balance
Ferrous Sulphate Heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	0.100	Trace iron
Cobalt Chloride Hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$)	0.100	Trace mineral; vitamin B12 synthesis
Calcium Chloride (CaCl_2)	0.100	Trace mineral; cell membrane function
Zinc Sulphate Heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$)	0.100	Trace mineral; enzyme cofactor
Copper Sulphate Pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)	0.010	Trace mineral; cytochrome oxidase
Aluminium Potassium Sulphate $12\text{H}_2\text{O}$ ($\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$)	0.010	Trace mineral
Boric Acid (H_3BO_3)	0.010	Trace mineral
Sodium Molybdate Dihydrate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$)	0.010	Trace mineral; nitrogenase cofactor
DI / Purified Water	to 1000 mL	Add nitrilotriacetic acid to ~500 mL water, adjust pH 6.5 with KOH, bring to 1000 mL, add remaining compounds one at a time

Solution B — Formula (per litre of final medium)

Ingredient	Amount	Function
Ferrous Sulphate Heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	20.0 g	PRIMARY ENERGY SOURCE — A. ferrooxidans oxidises $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ as sole energy-yielding reaction. Do NOT substitute or reduce.
DI / Purified Water	200 mL	Dissolve and filter-sterilise IMMEDIATELY. A. ferrooxidans cannot grow without adequate Fe^{2+} .

Preparation Protocol (Official ATCC 2039 Method)

Solution A — Step 1	Add nitrilotriacetic acid to ~500 mL DI water and adjust pH to 6.5 with KOH to prepare Wolfe's Mineral Solution. Bring to 1000 mL and add
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	remaining mineral compounds one at a time. Set aside 5.0 mL for use in Solution A.
Solution A — Step 2	Dissolve $(\text{NH}_4)_2\text{SO}_4$ (0.8 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (2.0 g), and K_2HPO_4 (0.4 g) in 800 mL DI water. Add 5.0 mL of Wolfe's Mineral Solution. Mix well.
Solution A — Step 3	Adjust pH to 2.3 with dilute H_2SO_4 . Verify with calibrated pH meter. DO NOT use HCl — chloride inhibits iron oxidation.
Solution A — Step 4	Filter sterilise Solution A through 0.22 μm membrane into a sterile vessel. DO NOT AUTOCLAVE.
Solution B — Step 5	Dissolve 20.0 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 200 mL DI water. Stir to dissolve quickly.
Solution B — Step 6	Filter sterilise Solution B IMMEDIATELY through 0.22 μm membrane. Use within 30–60 minutes. Do NOT store Solution B — Fe^{2+} oxidises rapidly in air.
Complete Medium	Aseptically combine sterile Solution A (800 mL) + sterile Solution B (200 mL). Mix gently. Final volume: 1 litre. A yellow precipitate is normal — it becomes darker as iron oxidises during incubation.
Incubation	Dispense as required. Incubate at 26–30°C aerobically with shaking (100–150 rpm) for 4–14 days.
DO NOT AUTOCLAVE	CRITICAL: Autoclaving oxidises $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$, precipitating iron hydroxide and destroying the medium. Filter sterilisation only for both solutions.

Storage & Stability

Solution A powder	15–25°C, dry, tightly sealed, protected from moisture. Shelf life: 2 years from manufacture.
Solution B ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	15–25°C, dry, tightly sealed, protected from moisture and air. Shelf life: 2 years. Do NOT store prepared Solution B.
Prepared medium	Use within 24 hours. Continuous iron oxidation makes long-term storage unsuitable.
Wolfe's Mineral Solution (prepared)	Store at 4°C, protected from light. Use within 6 months.

Literature References

1	ATCC. Medium 2039: Acidithiobacillus ferrooxidans Medium. American Type Culture Collection; current edition. atcc.org [PRIMARY REFERENCE — formula sourced directly from official ATCC PDF]
2	Silverman MP, Lundgren DG. Studies on the chemoautotrophic iron bacterium Ferrobacillus ferrooxidans. J Bacteriol. 1959;77(5):642–647. [Original 9K medium]

3	Wolin EA, et al. Wolfe's Mineral Solution. J Bacteriol. 1963;81:911–918. [Wolfe's Mineral Solution reference]
4	Kelly DP, Wood AP. Reclassification of some species of Thiobacillus. Int J Syst Evol Microbiol. 2000;50:511–516.
5	ISO 11133:2014. Microbiology of food and water — Performance testing of culture media. Geneva: ISO.

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