Nitrobacter sp.

25384™

Description

Strain designation: Nb-213
Deposited As: *Nitrobacter* sp.
Type strain: No

Storage Conditions

Product format: Freeze-dried

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and

will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:
ATCC Medium 480: Nitrobacter medium 203
Temperature: 26°C
Atmosphere: Aerobic
Incubation: With shaking

Handling Procedures

1. Rehydrate entire vial contents with 0.5 ml of #480 broth. Aseptically transfer this aliquot to 5.0 ml of the broth contained in a large test tube (20 X 150 mm).

2. Incubate tube at 26ºC, static, in the dark, and in a slanted position to increase surface area.

3. Monitor culture growth daily by assaying for the disappearance of NO_2 in the culture medium. Withdraw 0.1 ml from the culture and place in a small test tube. Add one drop of NO_2 Reagent A followed by one drop of NO_2 Reagent B, mixing after

each addition. Pink color development occurs within 10 minutes and is directly related to amount of NO_2 present. Initial tests will probably be dark magenta, reflecting the 20 mM NO_2 concentration of medium #480; but as culture grows and NO_2 is oxidized, color will become magenta, pink and finally clear. This process can take from 3 to 20 days, or possibly longer, depending on the viability of the stock.

4. When all NO_2 has been oxidized, inoculate a 250 ml flask containing 100 ml of medium #480 with the 5.0 ml tube culture. Wrap the flask in foil to protect from light and incubate at 26ºC with gentle shaking. Monitor NO_2 concentration daily.

5. When NO_2 is again depleted in the culture medium, either transfer the culture to fresh media or feed the culture by adding additional NO_2. A 10% inoculum is recommended for transfers. To feed cultures, aseptically add sterile $NaNO_2$ solution, returning NO_2 concentrations to approximately 10 mM (3.5 ml of 2% $NaNO_2$ per 100 ml culture). Cells should be harvested or sub-cultured to fresh media when cultures can utilize 10 mM NO_2 in less than 24 hours and cell numbers are greater than 10 cells per field at 1000X magnification.

6. Cells may be harvested by centrifugation. For long-term storage, 1.0 ml aliquots of concentrated cells can be frozen with glycerol as the cryoprotectant at a final concentration of 10%. The frozen vials should be stored at 70ºC or below for best preservation.

Notes

This item will not produce turbidity in broth. Growth can be detected by testing for the depletion of nitrite from the broth.

Reagent A:
Sulfanilic acid, 8 g
5N Acetic acid, 1.0 L

NO_2 Reagent B:
N,N-Dimethyl-1-naphthylamine, 6.0 ml
5N Acetic acid, 1.0 L

5N acetic acid consists of 1 part glacial acetic acid to 2.5 parts distilled water.

NO_2 Reagents A and B may also be purchased from Remel.

Additional information on this culture is available on the ATCC® web site at www.atcc.org.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Nitrobacter* sp. (ATCC 25384)

References

References and other information relating to this material are available at www.atcc.org.

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Revision

This information on this document was last updated on 2025-02-14

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