ATCC

Product Sheet

Nitrobacter sp. (ATCC® 25385™)

Please read this FIRST

Biosafety Level
1

Intended Use

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

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Nitrobacter sp.* (ATCC® 25385™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Designation: Nb-215 [Santa Cruz soil sample 5]
Deposited Name: *Nitrobacter* sp.

Propagation

Medium
ATCC® Medium 480: Nitrobacter medium 203

Growth Conditions
Temperature: 26.0°C
Atmosphere: With shaking

Propagation Procedure

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

NO_2 Reagent A: Sulfanilic acid, 8 g
5 N Acetic acid, 1.0 L
NO_2 Reagent B: N,N-Dimethyl-1-naphthylamine, 6.0 ml
Acetic acid, 1.0 L
The 5 N acetic acid consists of 1 part glacial acetic acid and 2.5 parts distilled water.
Additional information on this culture is available on the ATCC® web site at www.atcc.org.

References

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

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the

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ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

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