



Product Sheet

Nitrobacter winogradskyi (ATCC® 25391™)

Please read this **FIRST**



Storage Temp.
Frozen: -80°C or colder
Freeze-Dried: 2°C to 8°C
Live Culture: See Propagation Section



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 winogradskyi* (ATCC® 25391™)

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-255 [1]
Deposited Name: *Nitrobacter winogradskyi* Winslow et al.
Product Description: Type strain

Propagation

Medium

ATCC® Medium 480: Nitrobacter medium 203

Growth Conditions

Temperature: 26°C
Atmosphere: Aerobic

Propagation Procedure

1. Allow vial to thaw. Transfer the entire contents into 5.0 mL of #480 broth contained in a large test tube (20 X 150 mm).
2. Incubate tube at 26°C, static, in the dark, and in a slanted position to increase surface area.
3. Monitor culture growth daily by assaying for the disappearance of NO₂ in the culture medium. Withdraw 0.1 mL from the culture and place in a small test tube. Add one drop of NO₂ Reagent A followed by one drop of NO₂ Reagent B, mixing after each addition. Pink color development occurs within 10 minutes and is directly related to amount of NO₂ present. Initial tests will probably be dark magenta reflecting the 3 mM NO₂ concentration of medium #480; but as culture grows and NO₂ is oxidized, color will become magenta, pink and finally clear. This process can take from 3 to 20 days depending on the viability of the stock.
4. When all NO₂ 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 26°C with gentle shaking. Monitor NO₂ concentration daily.
5. When NO₂ is again depleted in the culture medium, either transfer the culture to fresh media or feed the culture by adding additional NO₂. A 10% inoculum is recommended for transfers. To feed cultures, aseptically add sterile NaNO₂ solution, returning NO₂ concentrations to approximately 10 mM (3.5 mL of 2% NaNO₂ per 100 mL culture). Cells should be harvested or sub-cultured to fresh media when cultures can utilize 10 mM NO₂ in less than 24 hours and cell numbers are greater than 10 cells per field at 1000X magnification.
6. Cells can 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%. Frozen vial should be stored at -70°C or below for best preservation.

Notes

Cells in #480 broth are motile coccobacilli arranged in singles.
When a strain is received as a test tube culture, transfer to fresh media upon arrival. (See step 4.)

NO₂ Reagent A:
Sulfanilic acid, 8 g
5N Acetic acid, 1.0 L

NO₂ 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.

NO₂ 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.

References

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

Biosafety Level: 1



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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.

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

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