



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**

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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](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
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## 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 NO<sub>2</sub> in the culture medium. Withdraw 0.1 mL from the culture and place in a small test tube. Add one drop of NO<sub>2</sub> Reagent A followed by one drop of NO<sub>2</sub> Reagent B, mixing after each addition. Pink color development occurs within 10 minutes and is directly related to amount of NO<sub>2</sub> present. Initial tests will probably be dark magenta reflecting the 3 mM NO<sub>2</sub> concentration of medium #480; but as culture grows and NO<sub>2</sub> 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 NO<sub>2</sub> 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 NO<sub>2</sub> concentration daily.
5. When NO<sub>2</sub> is again depleted in the culture medium, either transfer the culture to fresh media or feed the culture by adding additional NO<sub>2</sub>. A 10% inoculum is recommended for transfers. To feed cultures, aseptically add sterile NaNO<sub>2</sub> solution, returning NO<sub>2</sub> concentrations to approximately 10 mM (3.5 mL of 2% NaNO<sub>2</sub> per 100 mL culture). Cells should be harvested or sub-cultured to fresh media when cultures can utilize 10 mM NO<sub>2</sub> 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.)

NO<sub>2</sub> Reagent A:  
Sulfanilic acid, 8 g  
5N Acetic acid, 1.0 L

NO<sub>2</sub> 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<sub>2</sub> 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](http://www.atcc.org).

## References

References and other information relating to this product are available online at [www.atcc.org](http://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](http://www.atcc.org).

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