



Product Sheet

# *Nitrobacter sp.* (ATCC® 25385™)

Please read this **FIRST**



## 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™)

## 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 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 20 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, or possibly longer, 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 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 70°C or below for best preservation.

## Notes

NO<sub>2</sub> Reagent A: Sulfanilic acid, 8 g

5 N Acetic acid, 1.0 L

NO<sub>2</sub> 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](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

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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