### Designation
SG3902

### Deposited Name
Orenia salinaria Moune et al.

### Propagation

#### Medium
ATCC® Medium 2180: Orenia salinaria medium

#### Growth Conditions
- **Temperature:** 40.0°C
- **Atmosphere:** Anaerobic

#### Propagation Procedure
1. Sterilize the top of the Balch tube by spraying it with 70% ethanol and then flaming the top.
2. If needed exchange the gas in the Balch tube for 100% N\textsubscript{2}.
3. If the medium is pink (see discussion about resazurin) add 2.0 ml of reducing agent (3% cysteine, stock solution) per 100 ml of medium. Let the medium sit at room temperature for 10 to 20 minutes, until the resazurin becomes colorless, before inoculating.
4. When the Balch tube is ready to inoculate, open the vial according to enclosed instructions. Take an anaerobic 1.0 ml syringe (see discussion below) tipped with a 22 gauge needle and withdraw 0.5 ml of 2180 medium from the Balch tube and rehydrate the freeze dried pellet. Immediately place the rehydrated vial under a stream of sterile gas to maintain anaerobic conditions.
5. Using the same syringe transfer the rehydrated cell suspension back into a tube of #2180 broth. Plate 0.1 ml of the inoculated culture onto a non-selective agar medium and incubate aerobically at 37°C. Inoculate a nonselective anaerobic and aerobic broth. Transfer 0.5 ml of the rehydrated culture to a second tube of 2180 medium. Incubate the inoculated tubes at 37 to 45°C.
6. Growth should be detected in the #2180 broth within 24 to 48 hours. No growth should be detected on the aerobic plate, or in the nonselective aerobic or anaerobic broth.

### ANAEROBIC CONDITIONS:
- Balch tubes (available from Bellco Glass, Vineland, NJ) are specially designed for anaerobic work and use an aluminum crimp cap to hold a rubber stopper in place. Needles can easily be inserted through the stopper, and the tubes can be pressurized to 2 atm. Alternatively, serum vials may be used, or screw cap tubes with butyl rubber stoppers. In the latter case the stopper may be removed and the tube placed under a cannula system that dispenses sterile gas to maintain anaerobic conditions.
- Resazurin is a commonly used redox indicator that is pink when the redox potential is above 50 mv., and colorless when the redox potential is below 110 mv. i.e. highly reducing. Most strict anaerobes require this low redox potential for optimum growth.
- To obtain a fully reduced medium, it is necessary that the medium be anoxic and that a reducing agent be added. Common reducing agents are sodium sulfide, cysteine, dithiothreitol, and titanium citrate.
- Syringes can be made anaerobic by one of two methods. 1. Displace the dead space in the syringe with a sterile

### Notes

Cells appear as long rods in chains that are weakly motile. As the culture ages the cells will form spheroplasts and long irregular cells. When exposed to oxygen the cells quickly lose their integrity. In broth the cells form stringy clumps that will settle to the bottom. These clumps are easily re-suspended when the tube is shaken.

### 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

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

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