

503c: AMMONIFEX THIOPHILUS MEDIUM

| | | |
|-------------------|--------|----|
| Solution A | 922.00 | ml |
| Solution B | 50.00 | ml |
| Solution C | 10.00 | ml |
| Solution D | 1.00 | ml |
| Solution E | 10.00 | ml |
| Solution F | 10.00 | ml |

Sparge solution A with 100% CO₂ gas for 30 – 45 min to make it anoxic, distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Solutions C and F are autoclaved under 100% N₂ gas. Solutions D and E are prepared under 100% N₂ gas and sterilized by filtration. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 6.8, if necessary.

Solution A

| | | |
|--|--------|----|
| KH ₂ PO ₄ | 0.20 | g |
| NH ₄ Cl | 0.25 | g |
| NaCl | 1.00 | g |
| MgCl ₂ x 6 H ₂ O | 0.40 | g |
| KCl | 0.50 | g |
| CaCl ₂ x 2 H ₂ O | 0.15 | g |
| Trace element solution SL-10 | 1.00 | ml |
| Selenite-tungstate solution | 1.00 | ml |
| Sodium resazurin (0.1% w/v) | 0.50 | ml |
| Distilled water | 920.00 | ml |

Solution B

| | | |
|---------------------------------|-------|----|
| Na ₂ CO ₃ | 2.50 | g |
| Distilled water | 50.00 | ml |

Solution C

| | | |
|-----------------|-------|----|
| Na-formate | 2.00 | g |
| Distilled water | 10.00 | ml |

Solution D

| | | |
|---------------------------------------|------|----|
| Wolin's vitamin solution (10x) | 1.00 | ml |
|---------------------------------------|------|----|

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Solution E

| | | |
|--|-------|----|
| $\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{ H}_2\text{O}$ | 2.00 | g |
| Distilled water | 10.00 | ml |

Solution F

| | | |
|--|-------|----|
| $\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ | 0.30 | g |
| Distilled water | 10.00 | ml |

Trace element solution SL-10 (from medium 320)

| | | |
|--|--------|----|
| HCl (25%) | 10.00 | ml |
| $\text{FeCl}_2 \times 4 \text{ H}_2\text{O}$ | 1.50 | g |
| ZnCl_2 | 70.00 | mg |
| $\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$ | 100.00 | mg |
| H_3BO_3 | 6.00 | mg |
| $\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$ | 190.00 | mg |
| $\text{CuCl}_2 \times 2 \text{ H}_2\text{O}$ | 2.00 | mg |
| $\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$ | 24.00 | mg |
| $\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$ | 36.00 | mg |
| Distilled water | 990.00 | ml |

First dissolve FeCl_2 in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

| | | |
|--|---------|----|
| NaOH | 0.50 | g |
| $\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$ | 3.00 | mg |
| $\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$ | 4.00 | mg |
| Distilled water | 1000.00 | ml |

Wolin's vitamin solution (10x) (from medium 120)

| | | |
|----------------------------|---------|----|
| Biotin | 20.00 | mg |
| Folic acid | 20.00 | mg |
| Pyridoxine hydrochloride | 100.00 | mg |
| Thiamine HCl | 50.00 | mg |
| Riboflavin | 50.00 | mg |
| Nicotinic acid | 50.00 | mg |
| Calcium D-(+)-pantothenate | 50.00 | mg |
| Vitamin B ₁₂ | 1.00 | mg |
| p-Aminobenzoic acid | 50.00 | mg |
| (DL)-alpha-Lipoic acid | 50.00 | mg |
| Distilled water | 1000.00 | ml |