

1290: ALKALIPHILUS MEDIUM

| | | |
|-------------------|--------|----|
| Solution A | 950.00 | ml |
| Solution B | 25.00 | ml |
| Solution C | 1.00 | ml |
| Solution D | 25.00 | ml |

Dissolve ingredients of solution A, adjust pH to 8.0 with 1 N NaOH and boil medium for 1 min, then cool to room temperature under 100% N₂ gas atmosphere. Dispense solution A under same gas atmosphere in anoxic culture vessels (e.g., Hungate-type tubes) and autoclave. Prior to inoculation complete the medium by adding appropriate amounts of solutions B, C and D prepared under 100% N₂ gas and sterilized by filtration. Adjust pH of the completed medium to 8.0 with a sterile anoxic solution of 0.1 N HCl or 0.1 N NaOH, if necessary.

Solution A

| | | |
|--|--------|----|
| NaCl | 75.00 | g |
| MgSO ₄ x 7 H ₂ O | 8.80 | g |
| KCl | 1.00 | g |
| Yeast extract (OXOID) | 10.00 | g |
| Trypticase peptone (BD BBL) | 10.00 | g |
| Modified Wolin's mineral solution | 10.00 | ml |
| Distilled water | 940.00 | ml |

Solution B

| | | |
|-----------------|-------|----|
| D-Glucose | 2.50 | g |
| Distilled water | 25.00 | ml |

Solution C

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

Solution D

| | | |
|------------------|-------|----|
| Na-thioglycolate | 0.50 | g |
| Na-ascorbate | 0.50 | g |
| Distilled water | 25.00 | ml |

Modified Wolin's mineral solution (from medium 141)

| | | |
|--|------|---|
| Nitrilotriacetic acid | 1.50 | g |
| MgSO ₄ x 7 H ₂ O | 3.00 | g |

| | | |
|--|---------|----|
| MnSO ₄ x H ₂ O | 0.50 | g |
| NaCl | 1.00 | g |
| FeSO ₄ x 7 H ₂ O | 0.10 | g |
| CoSO ₄ x 7 H ₂ O | 0.18 | g |
| CaCl ₂ x 2 H ₂ O | 0.10 | g |
| ZnSO ₄ x 7 H ₂ O | 0.18 | g |
| CuSO ₄ x 5 H ₂ O | 0.01 | g |
| AlK(SO ₄) ₂ x 12 H ₂ O | 0.02 | g |
| H ₃ BO ₃ | 0.01 | g |
| Na ₂ MoO ₄ x 2 H ₂ O | 0.01 | g |
| NiCl ₂ x 6 H ₂ O | 0.03 | g |
| Na ₂ SeO ₃ x 5 H ₂ O | 0.30 | mg |
| Na ₂ WO ₄ x 2 H ₂ O | 0.40 | mg |
| Distilled water | 1000.00 | ml |

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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 |