496. DESULFOVIBRIO HALOPHILUS MEDIUM

Solution A:
\[
\begin{align*}
\text{Na}_2\text{SO}_4 & \quad 3.00 \text{ g} \\
\text{KH}_2\text{PO}_4 & \quad 0.20 \text{ g} \\
\text{NH}_4\text{Cl} & \quad 0.30 \text{ g} \\
\text{NaCl} & \quad 70.00 \text{ g} \\
\text{MgCl}_2 \times 6 \text{ H}_2\text{O} & \quad 3.00 \text{ g} \\
\text{KCl} & \quad 0.50 \text{ g} \\
\text{CaCl}_2 \times 2 \text{ H}_2\text{O} & \quad 0.15 \text{ g} \\
\text{Na-resazurin solution (0.1% w/v)} & \quad 0.50 \text{ ml} \\
\text{Distilled water} & \quad 930.00 \text{ ml}
\end{align*}
\]

Solution B:
Trace element solution SL-10 (see medium 320) \quad 1.00 \text{ ml}

Solution C:
\[
\begin{align*}
\text{Na}_2\text{CO}_3 & \quad 1.00 \text{ g} \\
\text{Distilled water} & \quad 20.00 \text{ ml}
\end{align*}
\]

Solution D:
\[
\begin{align*}
\text{Na-L-lactate} & \quad 2.50 \text{ g} \\
\text{Distilled water} & \quad 10.00 \text{ ml}
\end{align*}
\]

Solution E:
Vitamin solution (see medium 141) \quad 10.00 \text{ ml}

Solution F:
Selenite-tungstate solution (see medium 385) \quad 1.00 \text{ ml}

Solution G:
\[
\begin{align*}
\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O} & \quad 0.40 \text{ g} \\
\text{Distilled water} & \quad 10.00 \text{ ml}
\end{align*}
\]

Solution A is sparged with 80% N\textsubscript{2} and 20% CO\textsubscript{2} gas mixture to reach a pH below 6 (at least 30 min), then dispensed under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solutions B, D, F and G are autoclaved separately under 100% N\textsubscript{2} gas. Solution C is autoclaved under 80% N\textsubscript{2} and 20% CO\textsubscript{2} gas atmosphere. Solution E is filter-sterilized and gassed with 100% N\textsubscript{2} gas. To complete the medium appropriate amounts of solutions B to G are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 6.8 – 7.2.

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Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution freshly prepared under N₂ and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% (v/v) inoculum.