

## 641: DESULFOVIBRIO (MV) MEDIUM

This recipe contains strain-specific modifications for *Hydrogenispora ethanolica* DSM 25471 \*

Final pH: 7.0 - 7.2

Final volume: 1003 ml

NH <sub>4</sub> Cl	1.00	g
Na <sub>2</sub> SO <sub>4</sub>	2.00	g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	1.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	1.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
KH <sub>2</sub> PO <sub>4</sub>	0.50	g
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
<del>Na DL lactate</del>	<del>2.50</del>	<del>g</del>
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.10	g
<b>D-Glucose</b>	<b>2.00</b>	<b>g</b>
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, vitamins, lactate and sulfide), sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving complete the medium by adding vitamins (sterilized by filtration), lactate and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.

2. Note: Prior to inoculation 10-20 mg/l sodium dithionite (added from a 5% w/v solution freshly prepared under N<sub>2</sub> and filter-sterilized) can be added to the medium to stimulate growth at the beginning.

\* Replace lactate with 2.0 g/l D-glucose added to the autoclaved medium from a sterile anoxic stock solution.

### Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg

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CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl<sub>2</sub> 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
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	3.00	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> 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 <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml