

## Main sol. 962

Na <sub>2</sub> -9,10-anthraquinone-2,6-disulfonate (TCI A0308)	8.00	g
NH <sub>4</sub> Cl	0.33	g
KH <sub>2</sub> PO <sub>4</sub>	0.33	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.33	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.33	g
KCl	0.33	g
Yeast extract (OXOID)	0.05	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
NaHCO <sub>3</sub>	2.50	g
Na-acetate	3.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, acetate and vitamins), boil medium to dissolve AQDS, then cool to room temperature under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Add solid bicarbonate and adjust pH to 6.8 - 7.0. Dispense medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add acetate and vitamins from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.