

## Main sol. 1681

<b>Solution A</b>	50.00	ml
<b>Solution B</b>	50.00	ml
<b>Modified Wolin's mineral solution</b>	10.00	ml
<b>Wolin's vitamin solution</b>	10.00	ml
Sodium resazurin (0.1% w/v)	1.00	ml
Yeast extract	1.00	g
Polypeptone	10.00	g
NaHCO <sub>3</sub>	3.52	g
Colloidal chitin	5.00	g
Distilled water	890.00	ml

- Reducing agents:** Flush each solution under N<sub>2</sub> for 30 minutes, stopper and autoclave. L-Cysteine-HCL x H<sub>2</sub>O solution (10%): add 12.5 µl per 10 ml medium Na<sub>2</sub>S x 9 H<sub>2</sub>O solution (3%): add 40 µl per 10 ml medium.
- Colloidal chitin preparation:** Cut 5 gram commercial crab (shrimp) shell flakes into small pieces, and dissolve in 100 ml of 12M HCL while stirring in a fume hood at room temperature. Add chitin-HCL mixture slowly into pre-cooled water to obtain the colloidal chitin, centrifuge and wash in pre-cooled water till the colloidal chitin reaches a pH of 6 to 6.5. Mix 0.5% with MS medium as the sole carbon source. As an alternative commercial available chitin powder can be used.
- Prepare MS medium with chitin:** Dissolve the ingredients of MS medium (except NaHCO<sub>3</sub> and vitamins), boil medium under CO<sub>2</sub> until the resazurin indicator changes from pink to colourless. Cool down under CO<sub>2</sub>, add NaHCO<sub>3</sub> and adjust pH to 7.0. Switch to N<sub>2</sub> to prevent further lowering of the pH. Fill up into Hungate tubes under N<sub>2</sub> and autoclave. Before use add vitamins (sterilized by filtration) and reducing agents.