

## 193a. PHB/PYRUVATE MEDIUM

### Solution A:

Na <sub>2</sub> SO <sub>4</sub>	3.00	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
NH <sub>4</sub> Cl	0.30	g
NaCl	7.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	1.30	g
KCl	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
Selenite-tungstate solution (see medium 385)	1.00	ml
Poly[(R)-3-hydroxybutyric acid] (SIGMA)	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	930.00	ml

### Solution B:

Trace element solution SL-10 (see medium 320)	1.00	ml
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### Solution C:

Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Distilled water	30.00	ml

### Solution D:

Na-pyruvate	5.00	g
Distilled water	20.00	ml

### Solution E:

Vitamin solution (see medium 141)	10.00	ml
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### Solution F:

Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.40	g
Distilled water	10.00	ml

*Solution A* is sparged with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclaved. *Solutions B, D* and *F* are autoclaved separately under 100% N<sub>2</sub> gas. *Solution C* is autoclaved under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. *Solution E* is prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of *solutions B* to *F* are added to the sterile *solution A* in the sequence as indicated. Final pH of the medium should be 6.8 - 7.0.

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After inoculation, pressurize vessels with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 1 bar overpressure.

*Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized) may stimulate growth at the beginning. For transfers use 5 - 10% inoculum.*