193a. PHB/PYRUVATE MEDIUM

Solution A:
- Na$_2$SO$_4$ 3.00 g
- KH$_2$PO$_4$ 0.20 g
- NH$_4$Cl 0.30 g
- NaCl 7.00 g
- MgCl$_2$ x 6 H$_2$O 1.30 g
- KCl 0.50 g
- CaCl$_2$ x 2 H$_2$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

Solution C:
- Na$_2$CO$_3$ 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

Solution F:
- Na$_2$S x 9 H$_2$O 0.40 g
- Distilled water 10.00 ml

Solution A is sparged with 80% H$_2$ and 20% CO$_2$ 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$_2$ gas. Solution C is autoclaved under 80% N$_2$ and 20% CO$_2$ gas atmosphere. Solution E is prepared under 100% N$_2$ 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% H₂ and 20% CO₂ 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 N₂ and filter-sterilized) may stimulate growth at the beginning. For transfers use 5 - 10% inoculum.