195d: KNH MEDIUM

Solution A  
Na$_2$SO$_4$ 3.00 g  
KH$_2$PO$_4$ 0.20 g  
NH$_4$Cl 0.30 g  
NaCl 21.00 g  
MgCl$_2$ x 6 H$_2$O 3.00 g  
KCl 0.50 g  
CaCl$_2$ x 2 H$_2$O 0.15 g  
Yeast extract 0.50 g  
Ascorbic acid 0.10 g  
Trace element solution SL-10 1.00 ml  
Selenite-tungstate solution 1.00 ml  
Sodium resazurin (0.1% w/v) 0.50 ml  
Distilled water 950.00 ml

Solution B  
Na$_2$CO$_3$ 1.50 g  
Distilled water 30.00 ml

Solution C  
Na-acetate 0.70 g  
Distilled water 10.00 ml

1. 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 to 30% of their volume and autoclaved. Solution B is autoclaved separately under 80% N$_2$ and 20% CO$_2$ gas atmosphere. Solution C is autoclaved under 100% N$_2$ gas. Solutions D and E are prepared under 100% N$_2$ gas atmosphere and sterilized by filtration. To complete the medium solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.1 - 7.4.

2. After inoculation add 1 bar overpressure of sterile 80% H$_2$ and 20% CO$_2$ gas mixture.
**Solution D**

Wolin's vitamin solution (10x)  1.00 ml

**Solution E**

Tris-Cl buffer (1.0 M solution, pH 8.0)  7.20 ml
Na$_3$NTA x H$_2$O (0.5 M solution)  4.80 ml
TiCl$_3$ (15% w/v solution in HCl; Riedel-de Haen)  0.55 ml

Sterilize by filtration.

**Selenite-tungstate solution** (from medium 385)

NaOH  0.50 g
Na$_2$SeO$_3$ x 5 H$_2$O  3.00 mg
Na$_2$WO$_4$ x 2 H$_2$O  4.00 mg
Distilled water  1000.00 ml

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

HCl (25%)  10.00 ml
FeCl$_2$ x 4 H$_2$O  1.50 g
ZnCl$_2$  70.00 mg
MnCl$_2$ x 4 H$_2$O  100.00 mg
H$_3$BO$_3$  6.00 mg
CoCl$_2$ x 6 H$_2$O  190.00 mg
CuCl$_2$ x 2 H$_2$O  2.00 mg
NiCl$_2$ x 6 H$_2$O  24.00 mg
Na$_2$MoO$_4$ x 2 H$_2$O  36.00 mg
Distilled water  990.00 ml

First dissolve FeCl$_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 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$_{12}$  1.00 mg
p-Aminobenzoic acid  50.00 mg
<table>
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<th>Ingredient</th>
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<tbody>
<tr>
<td>(DL)-alpha-Lipoic acid</td>
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<tr>
<td>Distilled water</td>
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