

### 337: SYNTROPHOCOCCUS SUCROMUTANS MEDIUM

<b>Solution A</b>	921.00	ml
<b>Solution B</b>	50.00	ml
<b>Solution C</b>	1.00	ml
<b>Solution D</b>	25.00	ml
<b>Solution E</b>	10.00	ml
<b>Solution F</b>	1.00	ml

1. Sparge solution A with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 6.4 and dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solution C is prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. Solutions D, E and F are autoclaved separately under 100% N<sub>2</sub> gas. To complete the medium add appropriate amounts of solutions B - F to the sterile solution A in the sequence as indicated. Adjust pH of the complete medium to 6.4 - 6.8, if necessary.

2. Note: Rumen fluid may be replaced by supplementing the medium with 200 µg/ml of crude egg yolk phosphatidylcholine (SIGMA, type IX-E).

#### Solution A

<b>Mineral solution</b>	50.00	ml
<b>Trace element solution SL-10</b>	1.00	ml
<b>Clarified rumen fluid</b>	300.00	ml
Trypticase peptone (BD Bacto/BD BBL)	5.00	g
Na-formate	0.60	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	570.00	ml

#### Solution B

NaHCO <sub>3</sub>	2.50	g
Distilled water	50.00	ml

#### Solution C

<b>Seven vitamins solution</b>	1.00	ml
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#### Solution D

Lactose	5.00	g
Distilled water	25.00	ml

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### Solution E

L-Cysteine HCl x H <sub>2</sub> O	0.24	g
Distilled water	10.00	ml

### Solution F

Na <sub>2</sub> S x 9 H <sub>2</sub> O	78.00	mg
Distilled water	1.00	ml

### Mineral solution (from medium 335)

KH <sub>2</sub> PO <sub>4</sub>	10.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	6.60	g
NaCl	8.00	g
NH <sub>4</sub> Cl	8.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	1.00	g
Distilled water	1000.00	ml

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

HCl (25%)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl<sub>2</sub> in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

### Clarified rumen fluid (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N<sub>2</sub> gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

### Seven vitamins solution (from medium 503)

Vitamin B <sub>12</sub>	100.00	mg
p-Aminobenzoic acid	80.00	mg

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D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H <sub>2</sub> O	200.00	mg
Distilled water	1000.00	ml