

843. TREPONEMA AZOTONUTRICIUM MEDIUM

NaCl	1.00	g
KCl	0.50	g
MgCl ₂ x 6 H ₂ O	0.40	g
CaCl ₂ x 2 H ₂ O	0.10	g
NH ₄ Cl	0.30	g
KH ₂ PO ₄	0.20	g
Na ₂ SO ₄	0.15	g
Trace element solution (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Trypticase peptone (BD BBL)	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	2.50	g
Sugar mix (see below)	30.00	ml
Yeast autolysate (see below)	50.00	ml
Vitamin solution (see medium 503)	1.00	ml
Co-factors solution (see below)	20.00	ml
DL-Dithiothreitol (DTT)	0.40	g
Distilled water	900.00	ml

Dissolve ingredients (except bicarbonate, sugar mix, yeast autolysate, vitamins, co-factors, and DTT), sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume (e.g., 4.5 ml per Hungate-type tube) and autoclave. After sterilization add bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere and sugar mix, yeast autolysate, vitamins, co-factors and DTT from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and sterilized by filtration.

Check pH of complete medium and adjust to 7.2 – 7.4, if necessary.

Note: Basal medium and yeast autolysate have to be prepared freshly and cannot be stored for longer periods.

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Sugar mix:

Cellobiose	1.70	g
Sucrose	1.70	g
Maltose x H ₂ O	1.80	g
Trehalose	1.90	g
D-Xylose	0.75	g
Distilled water	30.00	ml

Yeast autolysate:

Suspend 14.0 g of dry baker's yeast (SIGMA YSC-1) in 50 ml distilled water and allow cells to autolyse for 48 h at 55°C. Thereafter, cell debris are removed by centrifugation (3.700 x g for 30 min) and the amber-colored supernatant is stored in aliquots at -20°C.

Co-factors solution:

Thiamine pyrophosphate	2.50	g
Pyridoxal-HCl	0.25	g
Pyridoxal phosphate	0.25	g
Ca-folate	0.05	g
β-NAD	0.05	g
Coenzyme A	0.05	g
FAD	0.05	g
Haemin (dissolved separately in 10 mM NaOH)	65.00	mg
Nicotinamide	25.00	mg
Folic acid	2.50	mg
Riboflavin	0.50	mg
Distilled water	1000.00	ml

Adjust pH to 7.0 with 1 N NaOH against indicator paper, filter sterilize and store under 100% N₂ gas atmosphere.

For [DSM 100394](#) replace sugar mix with 1.00 g/l xylan as substrate.