ATCC medium: 1191 *Clostridium thermocellum* medium (ATCC medium 1190) with 18.75 g filter paper substituted for the glucose

ATCC Medium 1190 (see below) with 18.75 g filter paper substituted for the glucose
When preparing tubed medium, Whatman #1 filter paper may be used. In this case, use one strip (8 X 70 mm) per tube of broth.

ATCC Medium 1190:
KH_2PO_4 .....1.5 g/L
Na_2HPO_4 . $12H_2O$.....4.2 g/L
NH_4Cl .....0.5 g/L
$MgCl_2$. $6H_2O$ .....0.18 g/L
Yeast Extract (BD 212750).....2.0 g/L
Glucose.....8.0 g/L
Vitamin Solution (see below).....0.5 ml/L
Wolfe's Modified Mineral Elixir (see below)....5.0 ml/L
Resazurin (0.1%).....1.0 ml/L
Reducing Solution (see below).....40.0 ml/L
Distilled deionized water to.....1.0 L

A note of caution: This medium contains sodium sulfide, and hydrogen sulfide production will occur, especially upon prolonged boiling. Hydrogen sulfide is hazardous and preparation of this medium should be done in a chemical fume hood.
Bring the medium to a boil while gassing with a mixture of 95% N_2, 5% H_2. Keep the solution boiling for several minutes until time the color turns from blue to reddish-pink. Add the reducing solution during continuous gassing and boiling. The pink color should disappear, indicating reduction. Boil briefly for reduction. Using anaerobic (Hungate) technique, dispense the medium in tubes flushed with N_2/H_2 gas mixture. Stopper the tubes with butyl rubber, secure and autoclave for 15 minutes.

Reducing Solution:
0.2 N NaOH.....200.0 ml
Na_2S . $9H_2O$ .....2.5 g
L-Cysteine . HCl.....2.5 g

Bring the sodium hydroxide solution to a boil and bubble with 95% N_2, 5% H_2 gas mixture. Allow the solution to cool and add the sodium sulfide and cysteine. Under anaerobic conditions, tube the solution, stopper with butyl rubber, and autoclave for 15 minutes.

Vitamin Solution:

Biotin	20.0 mg
p-Aminobenzoic acid	50.0 mg
Folic acid	20.0 mg
Pantothenic acid calcium salt	50.0 mg
Nicotinic acid	50.0 mg
Vitamin B12	1.0 mg
Thiamine . HCl	5.0 mg
Pyridoxine hydrochloride	100.0 mg
Thioctic acid	50.0 mg
Riboflavin	5.0 mg

Dissolve the vitamins in 500 ml of water. Add 0.05 ml of this solution per 100 ml of medium. Freeze the vitamin solution or store in a refrigerator in the absence of light.

Wolfe's Modified Mineral Elixir:

Nitrilotriacetic acid	1.5 g
$MgSO_4 . 7H_2O$	3.0 g
$MnSO_4 . H_2O$	500.0 mg
NaCl	1.0 g
$FeSO_4 . 7H_2O$	100.0 mg
$Co(NO3)_2 . 6H_2O$	100.0 mg
$CaCl_2$ (anhydrous)	100.0 mg
$ZnSO_4 . 7H_2O$	100.0 mg
$CuSO_4 . 5H_2O$	10.0 mg
$AlK(SO_4)_2$ (anhydrous)	10.0 mg
Boric acid	10.0 mg
$Na_2MoO_4 . 2H_2O$	10.0 mg
Na_2SeO_3 (anhydrous)	1.0 mg
Distilled water	1.0 L

Suspend the nitrilotriacetic acid in 500 ml water. Dissolve it by titrating with 2-3 N KOH until the pH is stabilized at 6.5. Add the rest of the ingredients and dissolve in the order they are listed. Finally, adjust the volume to 1.0 L For 100 ml of medium, use 0.5 ml of the mineral elixir.