

**ATCC medium: 1521 PYGV medium**

Peptone, Bacto (BD 211677).....	0.25 g
Yeast Extract (BD 212750).....	0.25 g
Mineral Salt Solution (see below).....	20.0 ml
Agar, Bacto (BD 214050), if needed.....	15.0 g
Distilled water.....	965.0 ml

Autoclave medium at 121C for 15 minutes. Cool to 50C and aseptically add:

2.5% glucose (filter-sterilized).....	10.0 ml
Vitamin Solution (see below) .....	5.0 ml

Adjust medium to final pH 7.5 with KOH.

*Mineral Salt Solution:*

Nitritilotriacetic acid.....	10.0 g
MgSO <sub>4</sub> . 7H <sub>2</sub> O .....	29.7 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O .....	3.34 g
Na <sub>2</sub> MoO <sub>4</sub> . 2H <sub>2</sub> O.....	12.67 mg
FeSO <sub>4</sub> . 7H <sub>2</sub> O .....	99.0 mg
Metals "44" (see below) .....	50.0 ml
Distilled water to.....	1.0 L

Dissolve nitritilotriacetic acid by neutralizing with KOH. Add remaining salts and adjust to pH 7.2 with KOH or H<sub>2</sub>SO<sub>4</sub>.

*Vitamin Solution:*

Biotin.....	4.0 mg
Folic acid.....	4.0 mg
Pyridoxine hydrochloride.....	20.0 mg
Riboflavin.....	10.0 mg
Thiamine . HCl.....	10.0 mg
Nicotinamide.....	10.0 mg
Calcium D-(+)-pantothenate.....	10.0 mg
Cyanocobalamin.....	0.2 mg
p-Aminobenzoic acid.....	10.0 mg
Distilled water.....	1.0 L

Filter-sterilize and store in dark at 5C.

*Metals "44":*

EDTA.....	0.25 g
ZnSO <sub>4</sub> . 7H <sub>2</sub> O .....	1.1 g
FeSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.5 g
MnSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.154 g
CuSO <sub>4</sub> . 5H <sub>2</sub> O .....	0.04 g
Co(NO <sub>3</sub> ) <sub>2</sub> . 6H <sub>2</sub> O.....	0.025 g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> . 10H <sub>2</sub> O .....	0.018 g
Distilled water.....	100.0 ml

Initially add a few drops of H<sub>2</sub>SO<sub>4</sub> to the distilled water to retard precipitation.