

ATCC medium: 2213 Biphasic trypanoplasma medium (SNB-9)

Biphasic Medium:

Solid phase (see below).....3.00 ml
Liquid overlay (see below).....0.75 ml

Solid Phase:

Neopeptone, DIFCO (BD 211681).....16.0 g
Agar, Bacto (BD 214050).....16.0 g
NaCl.....4.8 g
Sterile defibrinated rabbit or human blood...200.0 ml
De-ionized (DI) distilled water.....800.0 ml

Dissolve Neopeptone and NaCl in the distilled water. Add agar; mix gently and autoclave at 121C for 15 minutes. Cool to 45-50 C. Add sterile blood. Mix by swirling. Dispense in 3-ml aliquots to 16 X 125 mm screw-capped borosilicate test tubes.

Cool and solidify at a 12-degree slant in a refrigerator.

Liquid Overlay:

Neopeptone, DIFCO (BD 211681).....20.0 g
NaCl.....6.0 g
Vitamin Solution A (see below).....2.5 ml
Distilled water.....1.0 L

Mix the first two components and dissolve them in the distilled water. Autoclave at 121C for 15 minutes and cool. Add Vitamin Solution A.

Vitamin Solution A:

NaCl.....4.0 g
Riboflavin.....4.0 mg
Choline chloride.....40.0 mg
i-Inositol.....80.0 mg
Folic acid.....40.0 mg
Nicotinamide.....40.0 mg
Calcium D- pantothenate.....40.0 mg
Pyridoxal chloride.....40.0 mg
Thiamine chloride.....40.0 mg
DI distilled water.....100.0 ml

Add the components with mixing to the distilled water in the order listed and filter-sterilize. Cover the solid phase with a 0.75 ml overlay.