

**ATCC medium: 431 Trypanosome medium**

*Solid Phase:*

Beef extract.....3.0 g  
Peptone.....5.0 g  
NaCl.....8.0 g  
Agar.....15.0 g  
Distilled water.....1.0 L

*Liquid Phase (Locke's solution):*

NaCl.....8.0 g  
KCl.....0.2 g  
CaCl<sub>2</sub>.....0.2 g  
KH<sub>2</sub>PO<sub>4</sub>.....0.3 g  
Glucose.....2.5 g  
Distilled water.....1.0 L

Adjust pH of both phases to 7.2-7.4. Autoclave both phases at 121C for 15 minutes. Cool the solid phase mixture to about 45C and aseptically add 30% sterile, defibrinated, rabbit blood. Aseptically dispense in sterile 16 X 125 mm screw-capped test tubes in 5 ml amounts. Slant. After cooling the slants, aseptically dispense 3.0 ml of Locke's solution (Liquid phase) over each slant.