

**ATCC medium: 2706 CYE Medium with 200mcg/ml Natamycin**

Base Medium

Yeast Extract.....10.0g  
Charcoal\*..... 2.0g  
Agar.....17.0g  
DI Water.....720ml

Adjust pH to 6.90 +/- 0.05 using KOH. Autoclave at 121 C for 15 minutes.  
pH is critical. Cool to 50 C.

Aseptically add Solutions A, B, and C.  
Recheck pH and adjust if necessary. Note: allow sample to cool to room  
temperature before reading pH.

Keep charcoal in suspension when dispensing.

\*Activated Charcoal, Sigma C-5510

Solution A

L-Cysteine HCl.....0.4g  
DI Water.....10.0 ml  
Filter Sterilize.

Solution B

Fe-pyrophosphate.....0.25g  
DI Water.....10.0ml  
Filter Sterilize.

Note: Keep soluble Ferric pyrophosphate dry and in the dark. Do not  
use if the chemical loses its green color and becomes brown or yellow.  
Do not heat to dissolve.

Solution C

ACES Buffer (n-2-acetamido-2-aminoethane sulfonic acid)..... 10.0g  
DI Water.....250 ml  
Add chemical to water on stirrer. Add KOH (5 or 10 N) to adjust pH to 6.90  
+/- 0.05. The chemical will dissolve as the pH rises. Filter sterilize.

Solution D

Natamycin.....200 mg  
DI Water.....10.0 ml  
Filter Sterilize.