

81.CYE (Charcoal Yeast Extract) buffered medium

Yeast extract.....10.0 g
Charcoal (Sigma C-5510).....2.0 g
Agar.....17.0 g
Distilled water.....730.0 ml
Adjust pH to 6.90 +/- 0.05 using KOH.
Autoclave at 121C for 15 minutes.
pH is critical.
Cool to 50 C.
Aseptically add Solutions A, B, and C.
Check pH and adjust if necessary.

Note: Allow sample to cool to room temperature before reading pH. Keep charcoal in suspension when dispensing.

Solution A: L-Cysteine. HCl.....0.4 g Distilled water.....10.0 ml

Solution B: Ferric pyrophosphate.....0.25 g Distilled water.....10.0 ml 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)...10.0 g Distilled water.....250.0 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. Filtersterilize the pH-adjusted solution.