

Protocol I: Yeast Agar Medium with 5 mM acetamide solution (500 ml)

1. Mix in an autoclavable bottle:
 - a. 1M Tris-HCl Buffer Stock Solution (see Protocol II) 15 ml
 - b. YCB Medium Powder (supplied with kit) 5.85 g
 - c. Bacto agar (Becton Dickinson #214050) 10 g
 - d. Bring volume up to 495 ml with dH₂O
 - e. Autoclave 20 minutes at 121°C.Let cool to approximately 60°C.
2. Aseptically add 5 ml of sterile 100X acetamide solution.(supplied with kit)
3. Dispense into sterile disposable Petri dishes; Close plates and let sit at room temperature until solid, then invert and let sit for 12–18 hours to dry prior to use.

*Yeast carbon base (YCB) medium contains glucose and all nutrients needed to sustain growth of K. lactis GG799 Competent Cells except a simple nitrogen source. Cells can utilize acetamide as a source of nitrogen only after it is broken down to ammonia by acetamidase (the product of the amdS gene present in pKLAC2).
Acetamide should not be autoclaved.*

Protocol II: 1 M Tris-HCl Buffer Stock Solution (1 liter)

1. Solution A:

Dissolve 121.14 g Tris (American Bioanalytical #AB14042) in 800 µl dH₂O.
2. Adjust pH to 7.0 with the appropriate volume of concentrated HCl. Bring final volume to 1 liter with deionized water.
3. Autoclave and store at room temperature.