

## Yeast Medium Pack



1-800-632-7799  
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www.neb.com



B9017S 007130615061

# B9017S

**Yeast Carbon Base: 12 grams Lot: 0071306**

**Acetamide: 10 ml Exp: 6/15 Store at 4°C**

**Description:** The Yeast Medium Pack contains sufficient reagents needed to make 1 liter of Yeast Agar Medium containing 5 mM acetamide.

Yeast Agar Medium is used for acetamide selection of *K. lactis* cells that have been transformed with a pKLAC1-based expression vector (NEB #N3740). The medium contains glucose and all nutrients needed to sustain growth of *K. lactis* GG799 cells except a simple nitrogen source. Only transformed cells can utilize acetamide provided in the medium as a source of nitrogen after it is broken down to ammonia by acetamidase (the product of the *amdS* gene present in pKLAC1).

### Reagents Supplied:

Yeast Carbon Base	12 grams
100X acetamide solution (sterile)	10 ml

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

- Mix in an autoclavable bottle:
  - 1M Tris-HCl Buffer Stock Solution (see Protocol II) 15 ml
  - YCB Medium Powder (supplied with kit) 5.85 g
  - Bacto agar (Becton Dickinson #214050) 10 g
  - Bring volume up to 495 ml with dH<sub>2</sub>O
  - Autoclave 20 minutes at 121°C. Let cool to approximately 60°C.
- Aseptically add 5 ml of sterile 100X acetamide solution.(supplied with kit)
- 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)

- Solution A:  
Dissolve 121.14 g Tris (American Bioanalytical #AB14042) in 800 µl dH<sub>2</sub>O.
- Adjust pH to 7.0 with the appropriate volume of concentrated HCl. Bring final volume to 1 liter with deionized water.
- Autoclave and store at room temperature.

### Companion Products Sold Separately:

*K. lactis* Protein Expression Kit #E1000S  
*K. lactis* GG799 Competent Cells #C1001S 5 reactions  
pKLAC1 Vector #N3740S 20 µg

CERTIFICATE OF ANALYSIS

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