

Yeast Medium Pack



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



B9017S 009150517051

B9017S

Yeast Carbon Base: 12 grams Lot: 0091505

Acetamide: 10 ml Exp: 5/17 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 dH₂O
 - 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 dH₂O.
- 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:

<i>K. lactis</i> Protein Expression Kit #E1000S	
<i>K. lactis</i> GG799 Competent Cells #C1001S	5 reactions
pKLAC1 Vector #N3740S	20 µg

CERTIFICATE OF ANALYSIS

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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.

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