K. lactis YCT284 Competent Cells

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C1002S

5 Transformation Reactions Lot: 0041407
Store at –80°C Exp: 7/15

Description: Chemically competent Kluyveromyces lactis YCT284 (∆cts1) cells are a preferred host for secreting heterologous proteins carrying a chitin binding domain tag. YCT284 cells can be transformed with any linearized pKLAC-series expression vector. K. lactis strain YCT284 carries a selectable marker-free deletion of the KLLA0C04730g locus encoding an extracellular chitinase (KICts1p)

which can degrade chitin chromatography resin and compete for resin binding sites (1).

Transformation Protocol
The following steps should be conducted using aseptic technique. Care should be taken to ensure that pipet tips, tubes, solutions and deionized water are sterilized prior to use.

1. Thaw a tube of K. lactis YCT284 Competent Cells on ice. Add 620 µl NEB Yeast Transformation Reagent to the cells. Briefly shake or invert the tube until the solution is homogeneous. Do not vortex.

2. Add 1 µg of linearized pKLAC2 DNA containing the gene of interest to the cell mixture. Briefly shake or invert the tube to mix. Do not vortex. The total volume of transforming DNA should not exceed 15 µl.

3. Incubate the mixture at 30°C for 30 minutes.

4. Heat shock the cell mixture by incubation at 37°C for 1 hour in a water bath.

5. Pellet cells by microcentrifugation at ~7000 r.p.m. for 2 minutes and discard the supernatant.

Sterility: One tube of competent cells and 100 µl NEB Yeast Transformation Reagent were spread onto individual YCB Agar Medium plates containing 5 mM acetamide and incubated at 30°C for 3 days. No bacterial or fungal growth was detected.

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10. Remove 10, 50 and 100 µl of the cell suspension to separate fresh sterile 1.5 ml microcentrifuge tubes each containing 50 µl of sterile deionized water. Mix briefly and spread the entire cell mixture from each tube onto separate YCB Agar Medium plates containing 5 mM acetamide (see Media & Solutions). Incubate plates inverted at 30°C for 3–4 days until colonies form.

11. Streak or patch 10–20 individual colonies onto fresh YCB Agar Medium plates containing 5 mM acetamide. Incubate at 30°C for 1–2 days.

12. [OPTIONAL] Transformants can be tested to verify that they have correctly integrated the expression fragment.

13. [OPTIONAL] Correctly integrated transformants can be further screened to identify cells that have integrated multiple tandem copies of the expression fragment.

**Usage Notes:** Due to the high transformation efficiency of K. lactis YCT284 (Δcts1) Competent Cells, plating multiple dilutions of the cell mixture is necessary to ensure formation of plates with distinct single colonies. Growth time should not exceed 5 days as small colonies that lack an integrated expression fragment may form.

Plates containing colonies can be stored at 4°C for up to two weeks.

K. lactis YCT284 (Δcts1) Competent Cells may form small clumps when grown in liquid culture (1). This can obscure cell density measurements using light scattering techniques.

The deletion of locus KLLA0C04730g in YCT284 can be confirmed by PCR using the primers GGTCACCAGAAATACAAG and ATAAAAATATGA-TAAGGCTACACG to amplify a 2.0 kb diagnostic fragment.

The chitin binding domain (KICChBD) derived from the K. lactis secreted Cts1p chitinase (1) is not suitable for cytoplasmic expression and purification of KICChBD-tagged proteins.

**References:**


**Notice to Buyer/User:** K. lactis Competent Cells are a component of an expression system that was developed from basic research at New England Biolabs and DSM Biologics Company B.V. The buyer and user has a non-exclusive sublicense to use this system or any component thereof, including the K. lactis YCT284 Competent Cells, for RESEARCH PURPOSES ONLY. A license to use this system for manufacture of clinical grade material or commercial purposes is available from New England Biolabs, Inc., or DSM Biologics Company B.V.

U.S. Patent No. 7,517,671