K. lactis YCT284 Competent Cells

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YCT284

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

Strain: K. lactis strain YCT284 (Δcts1). No auxotrophies or genetic markers.

Reagents Supplied with Cells: NEB Yeast Transformation Reagent (NEB #M2570). Store at 4°C.

Quality Control Assays

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.

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5. Pellet cells by microcentrifugation at –7000 r.p.m for 2 minutes and discard the supernatant.
10. Remove 10, 50 and 100 µl of the cell suspension to separate fresh sterile 1.5 ml micro-centrifuge 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.

Patches of approximately 1.0 cm² are recommended. Plates containing patched cells may be stored at 4°C for up to 3 days prior to performing whole-cell PCR (optional steps 12, 13).

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 KLLA004730g in YCT284 can be confirmed by PCR using the primers GGTCACCAGAAATACAAG and ATAAAAATATGA-TAAGGCTACAG to amplify a 2.0 kb diagnostic fragment.

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

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