K. lactis YCT284 Competent Cells

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C1002S

5 Transformation Reactions **Lot: 0041206**

Store at –80°C **Exp: 12/12**

Description: Chemically competent *Kluyveromyces lactis* YCT284 (Δ*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 (KlCts1p) which can degrade chitin chromatography resin and compete for resin binding sites (1).

Store Competent Cells at –80°C
Once Thawed, Do Not Re-freeze

Strain: *K. lactis* strain YCT284 (Δ*cts1*). No auxotrophies or genetic markers.

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

Quality Control Assays

Competency: One microgram of linearized pKLAC1-*malE* was used to transform one tube of *K. lactis* YCT284 (Δ*cts1*) Competent Cells following the protocol provided. Greater than 1 x 10^4 colonies formed after a 3 day incubation at 30°C. **Use of cells beyond the expiration date may result in lower transformation efficiency.**

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.

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.
6. Resuspend the cell pellet in 1 ml sterile YPGlu medium (see Media & Solutions).
7. Pellet cells by microcentrifugation at ~7000 r.p.m for 2 minutes and discard the supernatant.
8. Resuspend the cell pellet in 1 ml YPGlu medium (see Media & Solutions) and transfer the cell mixture to a sterile culture tube. Incubate with shaking (250–300 r.p.m.) at 30°C for 3–4 hours.

Incubations shorter than 3 hours are not recommended due to a decline in transformation efficiency.

9. Transfer the cell mixture to a sterile 1.5 ml microcentrifuge tube. Pellet the cells by microcentrifugation at ~7000 r.p.m for 2 minutes and discard the supernatant. Resuspend the cell pellet in 1 ml sterile 1X PBS (see Media & Solutions).

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)

CERTIFICATE OF ANALYSIS

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.

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 (Δ*cts1*) 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 (Δ*cts1*) 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 ATAAAAATATGATAAGGCTACACG to amplify a 2.0 kb diagnostic fragment.

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

References:

1. Colussi, P.A. et al. (2005) *Appl. Environ. Microbiol* 71, 2862–2869.

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

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

Page 2 (C1002)