K. lactis Protease Deficient Competent Cell Sampler

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C1007S 006130214021

C1007S

5 Transformation Reactions **Lot: 0061302**

Store at –80°C **Exp: 2/14**

Description: Chemically competent *Kluyveromyces lactis* cells are suitable hosts for expressing proteins that have exhibited proteolysis in the parental GG799 background (NEB #C1001S). Proteolysis of secreted heterologous proteins in *K. lactis* is most often casued by aspartyl poroteases that are present in the secretory pathway. In many cases, a single aspartyl protease causes the majority of the detrimental protein degredation. The Protease Deficient Competent Cell Sampler contains a wild-type GG799 strain, as well as four protease deficient *K. lactis* strains, each having selectable marker-free deletion of a specific secretory pathway aspartyl protease gene. Each strain can be transformed with any linearized pKLAC-series expression vector, permitting a comparison of heterologous proein quality and yield in each background.

The Sampler contains one transformation each of five *K.lactis* strains. *K. lactis* GG799 is a wild-type expression strain. *K. lactis* YCT389 contains a selectable marker-free deletion of the KLLA0E0398g locus encoding an extracellular aspartyl protease (KlYps1p) that is homologous to *S.cervisaiae* Yps1p. *K. lactis* YCT390 carries a selectable marker-free deletion of the KLLA0F2208g locus encoding an extracellular aspartyl protease (Klyps7p) that is homologous to *S.cervisaiae* Yps7p. *K.lactis* strain YCT 569 carries a selectable marker-free deletion of the KLLA0D01507g locus encoding an extracellular aspartyl protease belonging to pfam00026. *K.lactis* strain YCT598 carries a selectable marker-free deletion of the KLLA0D15917g locus encoding an extracellular aspartyl protease that is homologous to *S.cervisaiae* Bar1p.

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

Reagents Supplied:

1 x 0.2 ml/tube of chemically competent *K. lactis* GG799 cells (NEB #C1001S)

1 x 0.2 ml/tube of chemically competent *K. lactis* YCT569 cells (NEB #C1003S)

1 x 0.2 ml/tube of chemically competent *K. lactis* YCT389 cells (NEB #C1004S)

1 x 0.2 ml/tube of chemically competent *K. lactis* YCT390 cells (NEB #C1005S)

1 x 0.2 ml/tube of chemically competent *K. lactis* YCT598 cells (NEB #C1006S)
(Store at –80°C)

5 ml of NEB Yeast Transformation Reagent
(Store at 4°C)

Quality Control Assays

Competency: One microgram of linearized pKLAC1-*malE* was used to transform one tube of each strain 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* 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.

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)

CERTIFICATE OF ANALYSIS

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.
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 cm^2 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* 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.

Growth of *K. lactis* Competent Cells carrying an integrated expression vector for protein expression should be performed in YPGal medium (1% yeast extract, 2% peptone, 2% galactose) supplemented with a final concentration of 38 mM $(NH_4)_2SO_4$. In the absence of 38 mM $(NH_4)_2SO_4$, YCT569, YCT389, YCT390 and YCT598 cells will grow slower than the GG799 parent strain (NEB #C1001).

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* Protease Deficient Competent Cell Sampler, 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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