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Product Information

Yeast Transformation Kit

Catalog Number **YEAST1**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The selection of plasmids in yeast is based on the use of auxotrophic mutant strains, which cannot grow without a specific medium component (an amino acid, purine, or pyrimidine). Transformation with a plasmid complementing the mutated gene enables the transformant to grow on medium lacking the required component. Yeast cells are made competent for transformation by incubation in a buffered lithium acetate solution. Transformation is then carried out by incubating the cells together with transforming DNA and carrier DNA in a solution containing polyethylene glycol (PEG).

The Yeast Transformation Kit utilizes the lithium acetate method that was first introduced by Ito, *et al*. (1983). The protocols provided include modifications suggested by Hill *et al.* (1991), Gietz *et al.* (1992) (protocol 1), and Elble (1992) (protocol 2).

Components

This kit is sufficient for over 100 standard transformations.

Transformation Buffer 100 mL
Catalog Number T0809
100 mM lithium acetate, 10 mM Tris-HCl, pH 7.6, and 1 mM EDTA

Plate Buffer 100 mL
Catalog Number P8966
40% PEG, 100 mM lithium acetate, 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA

Deoxyribonucleic acid 2 × 1 mL
from salmon testes, 10 mg/ml
Catalog Number D9156

Control Yeast Plasmid DNA pRS316, 10 µg
carrying the *ura* gene, ~1 mg/ml
Catalog Number C4959

Yeast Synthetic Drop-out Medium 1 g
Supplement Without Uracil
Catalog Number Y1501

Reagents Required but Not Provided.

(Catalog Numbers have been given where appropriate.)

- Sterile water
- Glucose, 50% solution, prepared from Catalog Number G7021, sterilized by 0.2 µm filtration
- Glycerol, 50% solution, prepared from Catalog Number G5516, sterilized by autoclaving
- DMSO, Catalog Number D8418 (optional)
- Bacteriological peptone
- Yeast extract, Catalog Number Y1625
- Bacteriological agar, Catalog Number A5306
- Yeast nitrogen base without amino acids, Catalog Number Y0626
- Yeast synthetic drop-out medium supplements without specific amino acids or pyrimidine:
  - Medium without leucine and tryptophan, Catalog Number Y0750
  - Medium without leucine, Catalog Number Y1376
  - Medium without histidine, Catalog Number Y1751
  - Medium without tryptophan, Catalog Number Y1876
  - Medium without histidine, leucine, tryptophan, and uracil, Catalog Number Y2001
- Yeast cells for use in transformation

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the kit at –20 °C. After thawing, store the Transformation Buffer, Plate Buffer, and Yeast Synthetic Drop-out Medium Supplement Without Uracil at room temperature.

Procedures

Preparation of Media Solutions

1. YPD medium and plates - Used to maintain yeast strains under non-selective conditions.

20 g/L	Bacteriological peptone
10 g/L	Yeast extract
20 g/L	Bacteriological agar (for plates)

   Add 960 mL of deionized water, autoclave **for 15 minutes**, and add 40 mL of a 50% sterile (0.2 μm filtered) solution of glucose.

2. Synthetic Complete (SC) drop-out plates - Used to select yeast strains **not requiring** a certain medium component (amino acid, purine, or pyrimidine).

0.8–1 g/L	Appropriate yeast synthetic drop-out medium supplement (Catalog Numbers Y1376, Y1751, Y1876, Y1501, Y0750, or Y2001)
6.7 g/L	Yeast nitrogen base without amino acids
20 g/L	Bacteriological agar

   Add 960 mL of deionized water, autoclave **for 15 minutes**, and add 40 mL of a 50% sterile (0.2 μm filtered) solution of glucose. Autoclaving the yeast-agar solution longer will cause plates to become soft. Alternatively, the medium can be autoclaved longer if the agar is autoclaved separately from the yeast nitrogen base.

Yeast Strains Maintenance

Yeast strains can be stored at 4 °C for 2–3 months on YPD or SC plates sealed with Parafilm®. For long term storage scrape a large inoculum from a freshly grown plate and resuspend in 1 mL of sterile 15% glycerol. Store at –70 °C.

To revive frozen yeast stocks, scrape frozen stock with a sterile toothpick or bacteriological needle, and streak on the appropriate plate (YPD or SC). Avoid thawing the stock. If the stock has thawed, vortex well before refreezing

Transformation Protocol 1

Preparation of competent cells

1. Inoculate yeast from YPD plate into 20 mL of YPD medium in a 100 mL sterile flask.
2. Grow overnight with shaking (200–250 rpm) at 30 °C. Culture should reach stationary phase (OD_{600} >2).
3. Dilute culture into 100 mL of YPD medium in a 500 mL sterile flask, so that the OD_{600} ~0.3. Grow with shaking at 30 °C for 3–6 hours. The OD_{600} of the culture should double at least once and should not pass 1.5.
4. Harvest cells by centrifugation at room temperature for 5 minutes at 5,000 rpm in a GSA or equivalent rotor.
5. Discard supernatant and resuspend cells in 50 mL of sterile water.
6. Repeat centrifugation as in Step 4.
7. Discard supernatant and resuspend in 1 mL of Transformation Buffer (Catalog Number T0809).

Note: Cells are now ready for transformation. It is best to use cells immediately, but they can be kept at 4 °C for 1 week with a gradual decrease in competence or glycerol can be added to 15% and cells kept frozen at –70 °C.

Plasmid Transformation

1. Set up the required number of sterile 1.5 mL microcentrifuge tubes, one for each transformation and one for a negative control.
2. Aliquot 10 μL of 10 mg/mL salmon testes DNA (Catalog Number D9156) into each tube.
3. Add 0.1 μg of the yeast plasmid DNA to be studied/Control Yeast Plasmid (1 μL of 0.1 mg/mL) into each tube.
   Note: The Control Yeast Plasmid DNA (Catalog Number C4959) is supplied. The plasmid serves as a positive control only for testing the transformation technique, using an *ura* deficient yeast strain and the supplied Yeast Synthetic Drop-out Medium Supplement Without Uracil (Catalog Number Y1501).
4. Add 100 μL of competent cells and vortex.
5. Add 600 μL of Plate Buffer (Catalog Number P8966) and vortex.
6. Incubate 30 minutes at 30 °C with shaking.
7. Optional: Add DMSO to 10%. This is not required, but will increase transformation efficiency 2 to 5-fold.
8. Heat shock for 15 minutes in a 42 °C heat block or water bath.
9. Spin 3 seconds in microcentrifuge and remove the supernatant. Do not spin longer as it will become hard to resuspend the cells.
10. Resuspend the cells in 500 μL of sterile water.
11. Plate 100 μL on appropriate SC selective plates without required supplement.
12. Incubate (face down) at 30 °C for 2–3 days until colonies appear.

Transformation Protocol 2 (Short)
This protocol is more rapid, but less efficient than Protocol 1. It is very convenient when only a small number of transformants are needed, but it is not recommended for transformations of libraries or other precious DNA.

1. Scrape a loop full of cells off a fresh plate (cells from a plate kept for 2 weeks at 4 °C will also be suitable) into a microcentrifuge tube.
2. Resuspend the cells in 0.5 mL of Transformation Buffer (Catalog Number T0809).
3. Spin 5 seconds in a microcentrifuge.
4. Remove the supernatant, leaving 50–100 µL of the buffer in tube.
5. Add 10 µL of 10 mg/mL salmon testes DNA (Catalog Number D9156) into each tube.
6. Add 1 µg of the yeast plasmid DNA to be studied/Control Yeast Plasmid (1 µL of 0.1 mg/mL) and vortex 10 seconds.
   Note: The Control Yeast Plasmid DNA (Catalog Number C4959) is supplied. The plasmid serves as a positive control only for testing the transformation technique, using an *ura* deficient yeast strain and the supplied Yeast Synthetic Drop-out Medium Supplement Without Uracil (Catalog Number Y1501).
7. Add 600 µl of Plate Buffer (Catalog Number P8966) and vortex.
8. Incubate 4–16 hours at room temperature.
9. Optional: Heat shock for 15 minutes in a 42 °C heat block or water bath.
10. Spin 3 seconds in microcentrifuge and remove the supernatant. Do not spin longer as it will become hard to resuspend the cells.
11. Resuspend cells in 500 µL of sterile water.
12. Plate 100 µL on appropriate SC selective plates without required supplement.
13. Incubate (face down) at 30 °C for 2–3 days until colonies appear.

References

1. Ito, H., *et al.*, Transformation of intact cells treated with alkali cations. J. Bacteriol., **153**, 163-8 (1983).
2. Hill, J., *et al.*, DMSO-enhanced whole cell yeast transformation. Nuc. Acids Res., **19**, 5791(1991).
3. Gietz, D., *et al.*, Improved method for high efficiency transformation of yeast. Nuc. Acids Res., **20**, 1425 (1992).
4. Elble, R., A simple efficient procedure for transformation of yeast. BioTechniques, **13**, 18-20 (1992).

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EM,NDH,SU,MAM 09/14-1