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A Geno Technology, Inc. (USA) brand name

Fast™ Yeast Transformation

(Cat. # GZ-1)

INTRODUCTION

Fast™-Yeast Transformation kit is designed to make yeast transformations easier and more efficient than protocols that are currently in wide usage. The competent yeast cells prepared with these reagents can be used immediately for transformation experiments or can be stored frozen at or below -70°C for direct use in the future. This method is suitable for both circular and linear plasmid transformations.
Reagents in this kit are designed for 120 regular or 600 micro-scale transformation experiments and are stable for 1 year at 4°C.

FEATURES

- High transformation efficiency: ≈10^5-10^6 transformants/µg plasmid DNA (circular).
- Frozen storage of competent cells for future use.
- Fast and Easy steps to make competent yeast cells within 10 minutes.
- Easy, single step transformation procedure that takes less than an hour.
- Broad spectrum (*S. cervisiae, C. albicans, S. pombe, Pichia pastoris*).
- Simple method for multiple plasmid transformations

ITEM(S) SUPPLIED (Cat. # GZ-1)

Description	Size
Wash Solution	60ml
Competent Solution	6ml
Transformation Solution	60ml

STORAGE CONDITIONS

The kit is shipped at ambient temp. Store all kit components at 4°C upon arrival.

STANDARD TRANSFORMATION PROTOCOL

Preparation of Competent Cells

1. Grow yeast cells at 30°C in 10ml YPD broth until mid-log phase (~5 x 10^6 - 2 x 10^7 cells/ml or OD_{600} of 0.8-1.0).
2. Pellet the cells at 500x g for 4 minutes and discard the supernatant.
3. Add 10ml Wash Solution to wash the pellet. Pellet the cells and discard the supernatant.
4. Add 1ml Competent Solution to resuspend the pellet.
5. At this point, the competent cells can be used for transformations directly or stored frozen at or below -70°C for future use. It is important to freeze the cells slowly. To accomplish this, either wrap the aliquoted cells in 2-6 layers of paper towels or place in a Styrofoam box before placing in the freezer. DO NOT use liquid nitrogen to snap-freeze the cells.

Transformation

This part of the procedure is the same for both frozen and freshly prepared competent yeast cells. If using frozen, thaw the cells at room temperature.

1. Mix 50µl of competent cells with 0.2-1µg DNA (in less than 5µl volume); add 500µl Transformation Solution and mix thoroughly.
2. Incubate at 30°C for 45 minutes. Mix vigorously by flicking with finger or vortexing (if appropriate for your DNA) 2-3 times during this incubation.
3. Spread 50µl -150µl of the above transformation mixture on an appropriate plate. It is unnecessary to pellet and wash the cells before spreading. Incubate the plates at 30°C for 2-4 days to allow for growth of transformants

   NOTE: *For transformations of C. albicans use freshly prepared competent cells; frozen cells sometimes give poor results.*

MULTIPLE PLASMID TRANSFORMATIONS

This protocol can generate enough transformants for most applications. If your work requires a large number of transformants as in library screening, use "Standard Transformation Protocol" and "Optimizing Your Conditions".

1. Prepare the competent cells as described in the "Standard Transformation Protocol".
2. The transformation can be performed in 96-well plates or tubes.
3. Add 10µl competent cells to DNA (0.2-1µg), mix by tapping or lightly vortexing; add 100µl Transformation solution and mix thoroughly.
4. Incubate at 30°C for 60-90 minutes. Mix vigorously 2-3 times during this incubation.
5. Directly spread the transformation mixture onto 1-2 plates.
6. Incubate the plates at 30°C for 2-4 days to allow for growth of transformants.

OPTIMIZING YOUR CONDITIONS FOR HIGHER EFFICIENCY

Cell growth conditions, strain differences, and other factors may influence the transformation efficiency. The following factors need to be considered if your experiments require high transformation efficiency:

Cell growth state

Cells used should be in mid-log phase. Early or late log-phase cells yield relatively fewer transformants.

Cell density

Optimal cell density is between 5 x 10^6 and 2 x 10^7 cells/ml (0.8-1.0 OD_{600}), but in most cases use of cultures with cell densities at the high end of the range greatly increases trans-formation efficiency in comparison to cultures with cell densities at the low end of the range.

The optimal cell density can also be achieved by re-suspending the cells in a smaller volume of Transformation Solution (for example, harvesting 10 ml cultures in low-density range and re-suspending in 0.5 ml of Transformation Solution instead of 1 ml as in the standard transformation protocol).

Incubation time after adding Transformation solution

Although an incubation time of 45 minutes is good for general purposes, transformation efficiency is much better with longer incubation times (up to 2-3 hours in most cases). Again, strains vary.

Medium used for plating

Not all commercially available media are created equal. The difference in transformation efficiency using different grade of media can be several fold. Our test results show that media from Difco are the most reliable.

DNA amount used

For transformation experiments using circular DNA such as 2µ based plasmids, we find that the efficiency stops increasing linearly as you increase the DNA above 1µg using the standard transformation protocol. For integrative transformation purity and amount of DNA used are important. Higher amount of linearized DNA is recommended to achieve the best results (up to 5µg of DNA can be used in the standard transformation protocol.

FREQUENTLY ASKED QUESTIONS

How long can I store my competent yeast cells below -70°C?
Presently, our data indicates that there is no loss of transformation efficiency after half a year of storage below -70°C. At this point, we expect the cell competency to be stable for at least 1 year.

How should I thaw the stored frozen competent cells?
Thaw at room temperature.

Does freezing and thawing affect the transformation efficiency?
We usually see a 10-30% increase of transformants after the first cycle of freezing. You can refreeze and thaw the competent yeast cells 3-4 times without noticeable effect on the transformation efficiency. Further freezing-thawing cycles adversely affect the transformation efficiency.

Do I need to incubate strictly at 30°C?
No. Temperatures between 30°C and 37°C are in the optimal temperature range. Incubation below or above this range greatly reduces the transformation efficiency.

Can DNA from restriction enzyme digestions be used without purification?
Yes. Different digestion buffers have only a slight effect on the transformation efficiency. You should try to keep the DNA volume in 5µl per transformation experiment by increasing the concentration of DNA in the digestion reaction

How much transformation mixture should be spread on a plate?
For most circular plasmid transformations, 50µl is enough. But if you use linearized DNA or use more than 1 selection marker, you can apply up to 200µl of transformation mixture on each plate to increase the number of transformants. The number of transformants increases linearly with the amount of transformation mixture applied to each plate.

Does this kit work on C. albicans, S. pombe, or Pichia pastoris?
Yes. Based on data from other labs and ours, this kit does work as well on *C. albicans, S. pombe,* or *Pichia pastoris* as well as on *S. cerevisiae.*

What is in this kit and how does it work?
The procedure utilized in this kit is designed, in some ways, similar to the lithium cation based method. No spheroplast step is involved. The mechanism probably involves some metabolic pathways that we do not fully understand.

Do I need to use plates with sorbitol?
No. Use any plate that is appropriate for your experiment.

NOTE: *Most of the above data are based on the testing of S. cerevisiae.*

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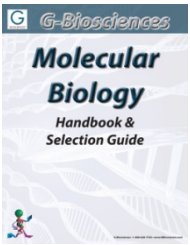

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