INSTRUCTIONS

Frozen-EZ Yeast Transformation II™

T2001

Highlights

- High transformation efficiency that yields approximately 10^5-10^6 transformants per 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. cerevisiae, C. albicans, S. pombe, Pichia pastoris*).
- Simple method for multiple plasmid transformations.

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ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

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GENERAL INFORMATION

Kit Contents:

Product	Storage Conditions
60 ml EZ 1 solution	0 - 4°C
6 ml EZ 2 solution	0 - 4°C
60 ml EZ 3 solution	0 - 4°C
Instruction sheet	
Reagents in this kit are designed for 120 regular or 600 micro-scale transformation experiments and are stable for 1 year at 4°C.	

Ordering Information:

Products	Cat No	Size
Frozen-EZ Yeast Transformation II ™ Reagents in this kit are designed for 120 regular or 600 microscale transformation experiments.	**T2001**	**1 kit**
For Individual Sale:		
Frozen-EZ Solution 1	T2002	60 ml
Frozen-EZ Solution 2	T2003	6 ml
Frozen-EZ Solution 3	T2004	60 ml

™ The Frozen-EZ Yeast Transformation II is a trademark of Zymo Research. For Research use only. Always wear protective gloves and eye protection. These reagents are intended for use by trained professionals. Further precautions should be taken according to your own company's regulations.

GENERAL DESCRIPTION

The **Frozen-EZ Yeast Transformation II**™ is designed to make yeast transformations easier and more efficient than protocols that are currently in wide usage. Version II further improves the efficiency and reliability of its predecessor. 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.

STANDARD TRANSFORMATION PROTOCOL

Note: For maximum transformation efficiency, please read "Optimizing Your Conditions for Higher Efficiency," pg. 3. If your experiment requires larger scale than the "Standard Transformation protocol" you can increase the volume in each step proportionally according to this procedure.

Preparation of Competent Cells

Grow yeast cells at 30°C in 10 ml YPD broth until mid-log phase (~5 x 10^6 - 2 x 10^7 cells/ml or OD_{600} of 0.8-1.0). The following steps are accomplished at room temperature.

1. Pellet the cells at 500 x g for 4 minutes and discard the supernatant.
2. Add 10 ml **EZ 1 solution** to wash the pellet. Repellet the cells and discard the supernatant.
3. Add 1 ml **EZ 2 solution** to resuspend the pellet.

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 aliquotted 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 stored (thawed at room temperature) and freshly prepared competent yeast cells.

1. Mix 50 µl of competent cells with 0.2-1 µg DNA (in less than 5 µl volume); add 500 µl **EZ 3 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 50-150 µl 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.

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

MICRO-SCALE PROTOCOL FOR MULTIPLE-PLASMID TRANSFORMATIONS

Note: For maximum transformation efficiency, please read "Optimizing Your Conditions for Higher Efficiency" below.

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" (page 2) and "Optimizing Your Conditions" below.

Preparation of competent cells: same as "Standard Transformation Protocol" (see page 2).

Transformation: This can be done either in 96-well plates or tubes.

1. Add 10 µl competent cells to DNA (0.2-1 µg), mix by tapping or lightly vortexing; add 100 µl **EZ 3 solution** and mix thoroughly.
2. Incubate at 30°C for 60-90 minutes. Mix vigorously 2-3 times during this incubation.
3. Directly spread the transformation mixture onto 1-2 plates.

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:

1) Cell growth state
Cells used should be in mid-log phase. Early or late log-phase cells yield relatively fewer transformants.

2) 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 transformation efficiency in comparison to cultures with cell densities at the low end of the range. The optimal cell density can also be achieved by resuspending the cells in a smaller volume of EZ 2 solution (for example, harvesting 10 ml cultures in low-density range and resuspending in 0.5 ml of EZ 2 instead of 1 ml as in the standard transformation protocol).

3) Incubation time after adding EZ 3 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, results vary according to strain used.

4) 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.

5) 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 1 µg 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 5 µg of DNA can be used in the standard transformation protocol).

6) Pre-warm solutions
Usually, it is not necessary to pre-warm all the solutions. For most purposes, cold solutions from the refrigerator will work fine. But, if your experiment requires maximum transformation efficiency, such as in library screening, the pre-warming of the solutions before the transformation experiment will result in higher efficiency. Calculate and measure the amounts of each solution that you will need to complete your experiment. Pre-warm these amounts to 20-37°C before doing experiments.

7) Outgrowth after transformation
Some selection markers and certain yeast strains need outgrowth step in rich medium for high efficiency transformation after Step 3 of Transformation, page 2. For this, add 4 volumes of YPD medium to the transformation mixture at the end of transformation, (2 ml YPD for the Standard Transformation, page 2) mix, incubate at 30°C for 2 hours. After this incubation, spin the cells down, resuspend the cells in 2 ml of water, spin the cells down again to wash the cells, resuspend the cells in original (0.5 ml for Standard Transformation Protocol) or less volume of water for plating on selection plates.

FAQ's

1. **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.
2. **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 *S. cerevisiae.*
3. **How long can I store my competent yeast cells below -70°C?**
   There is no loss of transformation efficiency after half a year of storage below -70°C. The transformation efficiency gradually starts to drop after 6 months.
4. **How should I thaw the stored frozen competent cells?**
   Thaw at room temperature.
5. **Do the frequencies of 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.
6. **In step 2 of the transformation, 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.
7. **Can I use DNA directly from restriction enzyme digestions without purification?**
   Yes. Different digestion buffers have only a slight effect on the transformation efficiency. You should try to keep the DNA volume in 5 µl per transformation experiment by increasing the concentration of DNA in the digestion reaction.
8. **How much transformation mixture should I spread on a selective plate?**
   For most circular plasmid transformations, 50 µl is enough. But if you use linearized DNA or use more than one selection marker, you can apply up to 200 µl 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.
9. **Do I need to use plates with sorbitol?**
   No. Use any plate that is appropriate for your experiment.
10. **Do I need to add carrier DNA?**
    No. There is no need for carrier DNA.

Note: Most of the data above is based on the testing of *S. cerevisiae.*