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Yeast Transformation Kit (Saccharomyces cerevisiae)

Product Number: YTK001

Shipping and Storage

Stored at -20℃, valid for at least one year. Except for Carrier DNA, it is stored at 4℃ and valid for 3 months. DMSO can also be stored at room temperature.

Component

Component	50T
Buffer A	105mL
Buffer B	5mL
Buffer C	30mL
Carrier DNA	500μL
DMSO	3.6mL

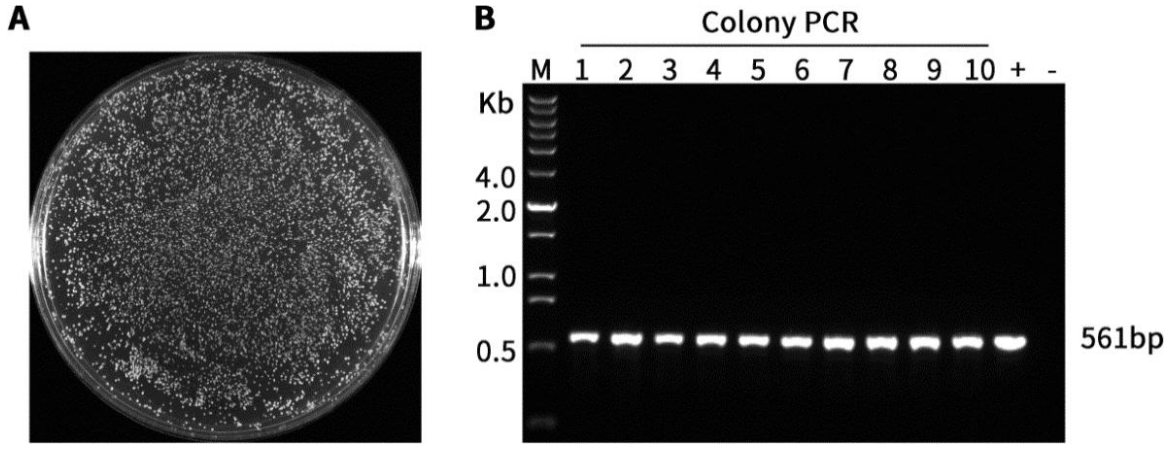
Description

Our company's Competitive Cell Preparation and Transformation Kit for Saccharomyces Cerevisiae is a rapid, convenient, and efficient kit for preparing highly active brewing yeast competent cells and performing plasmid transformation. This kit provides almost all the reagents required for the preparation and transformation of competent cells, except for the culture medium, without the need for dilution and preparation. The operation is simple and fast, and the preparation of competent cells can be completed within 30 minutes after yeast cultivation. It can be used for yeast hybridization experiments and yeast library construction experiments. When transforming brewing yeast, use the buffer solution provided in the reagent kit to culture yeast cells in the state to be transformed. Then mix the competent cells with the plasmid DNA and carrier DNA to be transformed, and incubate them with the transformation solution for transformation. Carrier DNA is a short linear single stranded DNA that promotes the entry of plasmids into yeast cells during the uptake of exogenous plasmid DNA, and also protects plasmids from degradation by DNA enzymes.

Saccharomyces cerevisiae is a single celled eukaryotic microorganism belonging to the genus Saccharomyces and the species Saccharomyces cerevisiae. Saccharomyces cerevisiae is a widely studied and used eukaryotic model with a genome of approximately 1.2×10^7bp. The nucleus contains 16 chromosomes and approximately 6000 ORFs, with only 4% of yeast genes having introns and a simple genetic background. Brewing yeast has growth characteristics similar to prokaryotes, making it easy to cultivate and perform genetic operations. It is a model eukaryotic organism, known as the "Escherichia coli" of eukaryotes. Brewing yeast can exist in a haploid state, making it easier to perform genotype phenotype analysis and efficient homologous recombination, making it easy to edit genome sequences for high-throughput genetic analysis. The expression system of brewing yeast has a certain degree of post-translational processing ability when expressing exogenous genes. The harvested exogenous proteins undergo folding processing and glycosylation modification to a certain extent, which is beneficial for maintaining protein activity and stability. Moreover, exogenous genes can be secreted and expressed in brewing yeast, and the secretion of expressed products outside the cell is not only beneficial for purification, but also avoids the accumulation of large amounts of products inside the cell.

During the transformation of brewing yeast, appropriate plasmids can be selected based on the nutritional deficiency type mutations of the strain. Generally speaking, if specific components of the culture medium (amino acids, purines, or pyrimidines) are lacking, mutant strains cannot grow. Using plasmids complementary to the mutant gene of the strain can enable the transformant to grow on the culture medium lacking specific components. Usually, transforming 1μg plasmid can produce>103 transformants, and the transformation efficiency may vary among different strains of brewing yeast.

Please refer to Figure 1 for the transformation effect of using our company's brewing yeast competent cell preparation and transformation kit.



The transformation effect diagram of Yeast Transformation Kit (Saccharomyces cerevisiae).

A. After 48 hours of cultivation in pGAL1,10-α factor-MCS-His-MCS-Flag URA vector using yeast INVSc1 competent cells prepared using Yeast Transformation Kit (Saccharomyces cerevisiae), plates were prepared. B. The colony in Figure A was subjected to electrophoresis using D7279 yeast colony PCR kit (enzymatic hydrolysis) after colony PCR. Mix 1μg of plasmid DNA and 10μL of heat denatured Carrier DNA, and add them to 100μL of competent cells prepared using this kit. Gently tap the bottom of the tube with your fingers and mix well; Add 600μL Buffer C and incubate in a 30℃ water bath for 30 minutes. After incubation, add 70μL DMSO and gently mix up and down. Incubate in a 42℃ water bath for 15 minutes, then immediately incubate in an ice bath for 3-5 minutes. Centrifuge at room temperature of approximately 12000-14000g for 10 seconds, discard the supernatant, gently add 100μL Buffer A and resuspend the bacterial cells. Finally, coat all onto nutrient deficient medium plates without Uracil and invert at 30℃ for 2-3 days until a single colony appears. Subsequently, PCR validation was performed using the D7279 yeast colony PCR kit (enzymatic hydrolysis). 561bp is the amplification band of the target gene fragment. M, DNA Ladder. 1-10, experimental group (PCR template is yeast single colony lysate),+, positive control; -Negative control. The actual usage effect of this product may vary due to differences in experimental conditions, materials, etc. The effects shown in the picture are for reference only.

This kit can be used multiple times, and small and medium packages can be prepared and used for competent cells that can undergo 50 and 250 transformations, respectively.

Protocol

1. Bacterial activation

Take glycerol yeast strains of brewing yeast stored at -80℃ and streak them on YPD agar medium. Invert and culture at 30℃ for 2-4 days to activate the strain.

2. Vaccination

Use an inoculation ring or sterile pipette to pick up a fresh yeast single colony and inoculate it into 10mL YPD liquid culture medium.

3. Cultivation

Incubate overnight at 28-30℃ and 250rpm.

4. Re inoculation and cultivation

Transfer the overnight cultured bacterial solution to 50ml YPD liquid medium for cultivation, with an initial OD600 between 0.2-0.3. Cultivate at 30℃ and 250-300rpm for 2-3 hours to achieve an OD600 of 0.4-0.6. Note: 4 tubes of 100μL agar can be prepared for every 10ml of bacterial solution. The following steps are applicable to 10ml bacterial solution, and the specific dosage of the sensory state can be prepared according to the actual situation.

5. Preparation of sensitive cells

5.1. When the OD600 of freshly cultured bacterial solution reaches 0.4-0.6, centrifuge at room temperature of 3000 × g for 3 minutes, discard the supernatant, and retain the bacterial cells.

5.2. Take 7mL of Buffer A and resuspend the collected bacterial cells by centrifugation. Centrifuge at room temperature at 3000 × g for 3 minutes, discard the supernatant, and retain the bacterial cells.

5.3. Take 400μL of Buffer B and resuspend the bacterial cells, incubate at room temperature for 10 minutes. After 10 minutes

of incubation, the yeast competent cells are ready, and then they can be divided into 1.5mL sterile centrifuge tubes according to 100μL each and transformed into yeast competent cells.

Note: It is best to use the prepared sensory state immediately; It can also be stored at 4℃ for one week, but the effect will gradually decrease; Alternatively, glycerol can be added to a final concentration of 15% and mixed well, then frozen in a -80℃ freezer for at least 3 months.

6. Conversion of brewing yeast

6.1. Mix 0.1-1μg plasmid DNA and 10μL heat denatured Carrier DNA by blowing and mixing, then add them to 100μL competent cells and mix gently by hand.

6.2. Add 600μL Buffer C solution, invert and mix well. Incubate in a water bath at 30℃ for 30 minutes.

6.3. After the previous step is completed, add 70μL DMSO, gently invert and mix well, incubate in a water bath at 42℃ for 15 minutes, and then immediately incubate in an ice bath for 3-5 minutes.

6.4. Centrifuge at room temperature of 12000-14000g for 10 seconds, discard the supernatant, and resuspend the bacterial cells in 100μL Buffer A.

6.5. Apply all the resuspended bacterial solution onto the corresponding screening medium plate and invert at 30℃ for 2-3 days until a single colony appears.

Note

1. When using Carrier DNA, the required amount of Carrier DNA can be taken out according to the conversion quantity, heated at 100℃ for 15 minutes, and then immediately placed in an ice bath. After 15 minutes, it can be used. The excess amount after use can be stored at -20℃, and the next time it is used, thaw the Carrier DNA and boil it again for denaturation. If the usage amount is small each time, it can be packaged appropriately before use to avoid repeated freezing and thawing. Heating treatment can shorten and denature Carrier DNA into single strands, while immediately placing it in an ice bath after heating can maintain the single stranded state. It is not recommended to heat all Carrier DNA at 100℃ for 15 minutes at once, as multiple treatments may cause the Carrier DNA to become too short.
2. The volume of the sample to be converted should not be too large, as a large sample volume can lead to a decrease in conversion efficiency.
3. Sensory cells are highly sensitive to temperature changes, and it is necessary to avoid temperature changes beyond the instructions for use.
4. Sensory cells are highly sensitive to mechanical forces. When adding the sample to be converted, handle gently and do not use a pipette to blow and mix thoroughly.
5. It is usually recommended to take some samples for conversion. In case of conversion failure, there are still samples left for further conversion.
6. All components of this reagent kit have undergone aseptic treatment. Please operate in a super clean bench during use, and seal and store after use to avoid contamination.
7. This product is only for scientific research by professionals and should not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residential areas.
8. For your safety and health, please wear lab coats and disposable gloves when operating.

Frequently asked questions

1. Factors affecting low conversion efficiency:

1.1. The conversion efficiency of brewing yeast varies due to differences in strains and cell growth conditions.

1.2. The use of high-purity plasmids can help improve transformation efficiency, and the transformation efficiency may vary among different vectors.

1.3. Adding DMSO before incubation in a 42℃ water bath will increase the conversion efficiency by 2 to 5 times.