Yeast Nucleus Extraction Kit

Cat: EX2910
Size: 50T/100T
Storage: 2-8℃ storage, valid for 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Component A: Yeast nucleus extract solution A	20mL	40mL	2-8℃
Component B: Yeast nucleus extract solution B	25mL	50mL	-20℃
Component C: Yeast nucleus extract solution C	25mL	50mL	2-8℃
Component D: Yeast nucleus preservation solution D	20mL	40mL	2-8℃

Note:

1. Extract B Avoid repeated freeze-thawing.
2. Use the reagent as soon as possible after unpacking!

Introduction:

Yeast nucleus extraction kit is suitable for extracting complete active nucleus from various yeast. The extraction process is simple and convenient. The prepared yeast core is of high purity, maintains natural activity and has little cross-contamination.

The extracted nuclei can be used for downstream applications such as nuclear function research or protein extraction.

Self-prepared reagents and instruments:

Centrifuge, oscillator, vortex mixer, pipette, refrigerator, ice box, PBS buffer, centrifuge tube, suction head, disposable gloves

Protocols:

First, notes for use:

1. The reagent in the rotating cap centrifuge tube should be centrifuged briefly before opening the cap, and the liquid on the inner wall of the cap should be thrown to the bottom of the tube to avoid the liquid spilling when opening the cap.
2. All reagents must be pre-cooled during the experiment; All utensils must be pre-cooled in a -20℃ refrigerator. The sample must be kept at a low temperature during the whole process.
3. Centrifuge speed has relative centrifugal force (RCF, ×g) and speed per minute (RPM, r/min) two ways to express, some centrifuges have RPM and ×g display switching, but some centrifuges do not have automatic switching function. Need to use the following formula for conversion: $g=r \times 1.118 \times 10^{-5} \times rpm^2$ (r is the effective centrifuge radius, the length in centimeters from the centrifuge axis to the center of the bottom of the centrifuge collection tube) For example: If the rotational speed is 3000rpm and the effective centrifugal radius is 10cm, then the relative centrifugal force (RCF, ×g) is$=10\times1.118\times10^{-5}\times3000^2=1006.2$ (×g).

Second, yeast nucleus extraction:

1. Yeast culture, centrifuge at 4℃, 1000×g for 5-10min, carefully absorb the medium, blot as much as possible, collect yeast precipitation.
2. Wash the yeast twice with PBS, sucking up as much supernatant as possible after each wash. [Note]: 1) Centrifuge at 1000×g for 5min.
3. Add 200μL yeast nucleus extract solution A per 100μL volume yeast sediment, mix well, and keep warm at 30℃ for 15min.
4. Centrifuge at 1000×g for 5-10min, discard the supernatant, and collect the yeast precipitate.
5. Wash the yeast once with 500μL PBS and centrifuge to collect the bacteria.

[Note]: 1) Centrifuge at 1000×g for 5min.

6. Add 300-500μL yeast nucleus extract soiution B into the yeast sediment and mix thoroughly.
   [Note]:
   1) Adjust the amount of extract liquid according to the number of yeast cells, generally 2-5 times the volume of bacteria.
   2) According to the volume of yeast bacteria every 100μL or 100mg wet weight bacteria to add 300-500μL extract solution.
7. Oscillate gently at 37°C or room temperature for 45-60min.
   [Note]:
   1) Use the lower speed of the oscillator/shaker, so that the extract can shake slightly.
   2) No oscillating conditions can also not oscillate, slightly extend the processing time of the extract, every few minutes with a pipette blow mix.
8. Centrifuge at 1000×g for 5-10min to collect the precipitate and discard the supernatant.
9. Wash the precipitate with 500μL PBS. Centrifuge to collect the precipitate.
   [Note]: 1) At 1000×g, centrifuge for 10min.
10. Add 500μL yeast core extract solution C to the precipitate, swirl at high speed for 15s to mix, then oscillate on an oscillator for 15-30min.
    [Note]:
    1) Use the lower speed of the oscillator/shaker, so that the extract can shake slightly.
    2) No oscillating conditions can also not oscillate, slightly extend the processing time of the extract, every few minutes with a pipette blow mix.
11. High speed vortex oscillation again for 5s, and then centrifuge at 4℃, 1000×g for 10min, discard the supernatant, precipitate the yeast nucleus.
12. The yeast nucleus was suspended with appropriate amount of reagent D and stored at 4℃ for further use or directly used in downstream experiments.
    [Note]:
    1) It can also be stored with other buffers according to the needs of downstream experiments.
    2) When the yeast core needs to be recovered again, centrifuge at 1000×g for 10 minutes to collect precipitation.

Notes:

1. Before the formal experiment, please select a few samples for pre-experiment, in order to optimize the experimental conditions and achieve the best experimental results.
2. Centrifuge the reagent in the screw cap microreagent tube briefly before opening the cap, and centrifuge the liquid on the cap and inside wall to the bottom of the tube to avoid reagent loss when opening the cap.
3. Do not mix with other brands of reagents, otherwise it will affect the use effect.
4. Contamination of the sample or reagent with bacteria or fungi or cross-contamination of reagents may result in false results.
5. It is best to use disposable suction heads, tubes, bottles, or glassware, and reusable glassware must be washed and thoroughly removed of residual cleaners before use.
6. All samples and exposed glassware should be disposed of in accordance with the prescribed procedure after the experiment is completed.