Yeast Golgi Extraction Kit

Cat: EX2920
Size: 50T/100T
Validity: 2-8℃ storage, valid for one year.

Kit Components:

Kit Components	50T	100T	Storage
Yeast Golgi extract solution A	20mL	40mL	2-8℃
Yeast Golgi extract solution B	25mL	50mL	2-8℃
Yeast Golgi extract solution C	20mL	40mL	2-8℃
Yeast washing solution D	50mL	100mL	2-8℃
Golgi preservation solution E	20mL	40mL	2-8℃

Note:

1. Store the kit at 2-8℃, and store the components according to the required conditions after opening the lid.
2. When the extraction solution B and yeast washing solution D are not used for a long time, they are stored at -20℃.
3. Please use the reagent as soon as possible after unpacking!

Introduction:

Golgi apparatus (Golgi bodies) are organelles composed of many flat vesicles whose main function is secretion. Also known as Golgi apparatus or Golgi complex; The Golgi apparatus is a highly polar organelle formed by several flat vesicles stacked together. They are usually located between the endoplasmic reticulum and the cell membrane and are arcuated or hemispherical, with the convex side facing the endoplasmic reticulum called the forming face or cis face. The concave side facing the plasma membrane is called the mature face or the opposite (trans face). There are some large or small transport vesicles on both sides of the face and the opposite side. In polar cells, the Golgi is often abundant in the cytoplasm of the secretory end. Because it looks very similar to the smooth endoplasmic reticulum, some scientists believe that it is evolved from the smooth endoplasmic reticulum.

The main function of the Golgi apparatus is to process, classify and package the proteins synthesized by the endoplasmic reticulum, and then send them to specific parts of the cell or secrete them outside the cell. The enzymes in the Golgi apparatus are mainly glycosyltransferase, sulfo-glycosyltransferase, oxidoreductase, phosphatase, protein kinase, mannosidase, transferase and phospholipase.

This kit provides a complete set of reagents suitable for the extraction of golgi bodies from various yeast samples. The extraction process is simple and convenient.

This kit cannot be used for Golgi extraction of frozen yeast samples.

The complete Golgi apparatus extracted by this kit can be used for downstream Golgi function study, or for protein cleavage to extract Golgi proteins.

Self-prepared reagents and instruments:

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

Protocols:

First, notes for use:

1. Please centrifuge the reagent in the rotating cap centrifuge tube briefly before opening the cap,

and throw the liquid on the inner wall of the cap to the bottom of the tube to avoid the liquid spilling when opening the cap.

2. All reagents in the process of the experiment must be pre-cooled; All appliances must be pre-cooled in the -20℃ refrigerator. The sample must be kept at a low temperature during the whole process.

Second, yeast golgi extraction:

1. Yeast culture, centrifuge at 4°C, 1000×g for 5-10min, carefully absorb the medium, blot as dry as possible, collect yeast precipitates.
2. Wash the yeast twice with PBS, sucking up as much supernatant as possible after each wash.
   [Note]: Centrifuge 1000×g for 5min.
3. Add 400μL of yeast golgi extract solution A to every 100μL volume or 100mg of wet weight yeast sediment, mix well, and keep warm at 30°C for 15min.
   [Note]: The amount of yeast is adjusted according to the experimental situation, and the amount of lysate per time is not certain.
4. Centrifuge at 1000×g for 5-10min to collect yeast precipitates.
5. Wash the yeast twice with 300μL yeast washing solution D and centrifuge to collect the yeast.
   [Note]: Centrifuge 2000×g for 5min
6. Add 500μL yeast golgi extract solution B to the yeast sediment and mix thoroughly.
   [Note]:
   ① According to the amount of yeast cells to adjust the amount of extract, generally add bacteria volume of 2-5 times can be.
   ② Add 250-500μL of extract solution according to 100ul or 100mg of wet heavy bacteria of yeast body volume.
7. Gently oscillate at 37°C or room temperature for 60-90min.
   [Note]:
   ① Use the lower speed of the oscillator/shaker, the extraction liquid can be slightly shaken. No oscillating conditions can also not oscillate, in the middle of every few hours with the pipette blow mixing can be.
   ② The processing time of different yeast samples varies greatly. According to the difficulty of downstream cell lysis adjustment, if the precipitation is not significantly reduced after treatment with downstream reagent C, it is necessary to extend the processing time of this step. It can be extended to 2 hours.
8. Centrifuge at 2000×g for 5-10min, discard the supernatant, and collect the precipitation.
9. Wash the precipitate twice with PBS. 2000×g centrifuge to collect the precipitate.
10. Add 400μL yeast golgi extract solution C to the precipitation, mix well, and then oscillate on the oscillator for 10-30min.
    [Note]:
    ① Use the lower speed of the oscillator/shaker, the extraction liquid can be slightly shaken. No oscillating conditions can also not oscillate, in the middle of every few hours with the pipette blow mixing can be.
    ② The precipitate of the next step after the treatment of reagent C should be reduced, otherwise the treatment time should be extended.
11. Centrifuge at 4°C, 500×g for 5min, discard the precipitation and collect the supernatant.
12. Centrifuge the supernatant at 4°C, 3000×g, for 10min. Discard the precipitation and collect the supernatant.

13. Centrifuge the supernatant at 5000×g at 4℃ for 10min. Discard the precipitation and collect the supernatant.
14. Centrifuge the supernatant at 20000×g at 4℃ for 20min. Discard the supernatant and leave to precipitate.
15. Add 400μL golgi preservation solution E to the precipitation and mix well.
16. Centrifuge at 20000×g at 4℃ for 30min. Discard the supernatant and collect the precipitation. That is, yeast golgi sample was obtained.
17. according to the needs of the downstream experiment, the precipitation is re-suspended with the corresponding buffer solution. Put in the refrigerator for use or directly for downstream experiment.

Notes:

1. Before the formal experiment, please select several samples for pre-experiment to optimize the experimental conditions and achieve the best experimental results.
2. The reagent in the screw cap trace reagent tube should be centrifuged briefly before opening the cap, and the liquid on the cap and inner wall should be centrifuged to the bottom of the tube to avoid the loss of the reagent when opening the cap.
3. It is prohibited to 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 lead to wrong results.
5. It is best to use disposable suction heads, tubes, bottles or glassware, and reusable glassware must be washed and thoroughly removed before use.
6. After the completion of the experiment, all samples and utensils in contact should be disposed of in accordance with the prescribed procedures.