

## Yeast Total Protein Extraction Kit

**Cat:** BC3780

**Size:** 50T/100T

**Storage:** Store according to storage conditions, shelf life of 1 year.

### Kit Components:

| Name                      | 50T     | 100T   | Storage                |
|---------------------------|---------|--------|------------------------|
| Wall Breaking Solution I  | 25mL    | 50mL   | 2-8°C                  |
| Wall Breaking Solution II | 25mL    | 50mL   | 2-8°C                  |
| Lysate                    | 25mL    | 50mL   | 2-8°C                  |
| β-mercapto reducing agent | 0.625mL | 1.25mL | 2-8°C, away from light |
| Protease inhibitor(100×)  | 0.25mL  | 0.5mL  | -20°C, away from light |
| PMSF(100×)                | 0.25mL  | 0.5mL  | -20°C, away from light |

### Protocols(for reference only):

**Note: β-sulphydryl reducing agent, protease inhibitor (100×), PMSF(100X) These reagents are centrifugated with a palm centrifuge before use.**

1. Take yeast thallus of OD600=1.0, centrifuge at 12000rpm at 4°C for 5min, and discard the supernatant.
2. Weigh the yeast thallus, 500μL wall-breaking solution I was added to the precipitation according to the proportion(50mg: 500μL), the bacteria were suspended, and incubated on ice for 5min.
3. Centrifuge at 12000rpm at 4°C for 2min, discard the supernatant, add 500μL wall-breaking solution II to the precipitation, resuspend the bacteria, and incubate on ice for 5min.
4. Add 5μL PMSF, 5μL protease inhibitor and 12.5μL β-mercapto reducing agent into the 500μL lysate; Mix well, place on ice for use(prepare appropriate amount of lysate according to the actual dosage), ready to use.
5. Centrifuge at 12000rpm for 2min at 4°C, discard the supernatant, and add 500μL the above-mentioned lysate prepared in advance to the precipitation. Shake at room temperature for 40min~60min, or boil for 5min.
6. After cracking, centrifuge at 12000rpm at 4 °C for 10min. The supernatant was collected for subsequent experiments.

### Notes:

1. Lysate may precipitate out at low temperature, 37°C water bath heating until precipitate completely dissolved before use.
2. β-mercapto reducing agent may be viscous at low temperature, and can be used after being restored to room temperature.
3. The extracted protein solution contains reducing agent. If you want to measure the

concentration, please precipitate the protein first, and then re-dissolve the protein with PBS before testing, otherwise the result will be affected.

4. According to every 50mg wet weight add 500  $\mu$ L wall breaking solution and cracking solution, the amount of bacteria can not be too much, otherwise it will make the bacteria cracking is insufficient.
5. The cracked yeast protein solution should be stored in the refrigerator at  $-80^{\circ}\text{C}$ .
6. The concentration of salt ions in the lysate is high, and it can be used after dialysis desalting if necessary.
7. If phosphorylated proteins are extracted, phosphatase inhibitors need to be added.
8. This reagent can only be used in vitro experimental research experiments, can not be used in clinical, therapeutic and animal in vivo experiments, etc., the resulting consequences, we will not be responsible.