

# Yeast Total Protein Extraction Kit

Cat No.: BC3780

Size: 50T/100T

Validity term: At least 1 year.

## Kit contents

Component	50T	100T	Storage
Yeast lysis buffer I	25ml	50ml	2-8 °C
Yeast lysis buffer II	25ml	50ml	2-8 °C
Lysis buffer	25ml	50ml	2-8 °C
$\beta$ -Mercaptoethanol	0.625ml	1.25ml	2-8 °C, avoid light
Protease inhibitor(100 $\times$ )	0.25ml	0.5ml	-20 °C, avoid light
PMSF(100 $\times$ )	0.25ml	0.5ml	-20 °C, avoid light

## Protocol

**\*Before using:  $\beta$ -Mercaptoethanol, Protease inhibitor(100 $\times$ ) and PMSF(100 $\times$ ) should be spinned briefly in a microcentrifuge.**

1. When the OD600 of yeast liquid is up to one, centrifugate it at 4°C, 12000rpm for 5 min, then discard supernatant.
2. Weigh the cells. Each 50mg cells should be added 500 $\mu$ L Yeast lysis buffer I. Resuspend the cells and incubate on ice for 5min.
3. Centrifugate the cells at 4°C, 12000rpm for 2 min and discard the supernatant. Add 500 $\mu$ L Yeast lysis buffer II to the cells. Resuspend the cells and incubate on ice for 5min.
4. Add 5 $\mu$ L of PMSF(100 $\times$ ), 5  $\mu$ L of Protease inhibitor(100 $\times$ ), 12.5 $\mu$ L of  $\beta$ -Mercaptoethanol into 500 $\mu$ L of Lysis buffer, mix well and put it on ice. Prepare the Lysis buffer according to actual dosage before use.
5. Centrifugate the cells at 4°C, 12000rpm for 2 min and discard the supernatant. Add 500 $\mu$ L Lysis buffer prepared in the previous step into the cells and resuspend it. Shock at room temperature for 40min~60min, or boil for 5min.
6. After the lysis is completed, centrifugate at 4°C, 12000rpm for 10 min. Collect the supernatant for subsequent experiments

## Note

1. The precipitate may appear in the lysis buffer at low temperature. It should be heated at 37 °C on water bath until the precipitate disappears.
2. Allow the  $\beta$ -Mercaptoethanol to return to room temperature before use, because it may be thick at low temperature.

3. The extracted protein solution contains  $\beta$ -Mercaptoethanol. If you want to measure the concentration, please precipitate the protein first, and then re-dissolve the protein with PBS before you measure it, otherwise it will affect the result.
4. Add 500 $\mu$ L of Yeast lysis buffer and Lysis buffer per 50 mg wet weight, the amount of cells should not be too much, otherwise it will cause insufficient lysis of cells.
5. The yeast protein liquid should be stored in the refrigerator at -80 °C.
6. The salt ion concentration in the Lysis buffer is high, and it can be used after dialysis desalination if necessary.
7. Phosphatase inhibitors should be added if you extract the phosphorylated proteins.
8. This reagent can only be used for in vitro experiments and scientific research experiments, and not for clinical, therapeutic and animal in vivo experiments, etc.