



PRODUCT INFORMATION

Yeast Protein Extraction Kit (Cat# PEY-1500)

Production Information for PEY-1500:

Introduction:

The kit provides optimized reagents and buffers for efficient total protein extraction from yeast strains. The kit contains EDTA, CaCl₂, DTT, protease inhibitors cocktail and a special reagent to break cell wall. The extracted proteins maintain biological activities and suitable for downstream assays including SDS-PAGE, Western blotting enzyme analysis, 2D gel electrophoresis and others. The kit is sufficient for 1,500 mg wet yeast cells.

Components	PEY-1500
Isoosmotic Buffer	75 ml
Snailase Storage Buffer	250 µl
Hypoosmotic Buffer	25 ml
PMSF	4 mg

Content:

The kit is made of isoosmotic buffer, snailase, hypoosmotic buffer, and PMSF protease inhibitor, and can be used for 50 times.

Protocol:

- 1 Cultivate the yeast strain in suitable medium at 30 or 28 °C until the OD₆₀₀ value of yeast density is about 1.0.
- 2 Centrifuge at 6,000 x g (8,000 rpm) for one minute, discard supernatant, and keep yeast paste and weigh the weight of wet paste
- 3 Per 70 mg of wet yeast paste, add 500 µl of Isoosmotic Buffer, 5 µl of Snailase Storage Buffer and 1 µl mercaptoethanol, pipet the mixed solution up and down to fully suspend yeast.
- 4 Incubate at 37°C for one hour, invert occasionally for more than three times.
- 5 Centrifuge at 4,000 x g (5,000 rpm) for one minute, discard supernatant and save precipitates.
- 6 Wash the precipitates with 500 µl of Isoosmotic Buffer, then centrifuge at 4,000 x g (5,000 rpm) for one minute, discard supernatant and save protoplasmic precipitates.
- 7 Repeat step 6.
- 8 Add 500 µl of Hypoosmotic Buffer and 0.5 µl of PMSF solution (PMSF is dissolved in 30 µl of isopropanol), vortex, and keep in icebox at -20 °C for 30 minutes, thaw it at room temperature. Repeat the freeze-thaw procedure once more.
- 9 Use the lysed solution for Western Blot, SD-PAGE, 2D Electrophoresis and immunoprecipitation experiments or store at -20 °C.

Note:

- 1 Per 70 mg wet yeast, add 500 µl of Isoosmotic Buffer, 5 µl of Snailase Storage Buffer, too much yeast will lead to incomplete cell wall removal.
- 2 Store the lysed solution at -20 °C for further experiments.

Storage:

Isoosmotic Buffer, Hypoosmotic Buffer and PMSF can be stored at 4 °C. The Snailase Storage Buffer must be stored at -20 °C.