

Minute™ Total Protein Extraction Kit for Microbes with Thick Cell Walls

Catalog number: YT-015

Description

Protein extraction from cell wall-containing microbes is a frequent procedure in bio-research Labs. The methods for protein extraction from these microbes are usually harsh, tedious and not sufficiently reliable. YT-015 provides an instrument-free, rapid and gentle way for extracting proteins from microbes with thick and strong cell walls. These microbes include but not limited to yeast, filamentous fungus, gram positive and negative bacteria, insect eggs and microalgae. This kit contains optimized denaturing and native protein extraction buffers for user to choose from. Unlike many other methods of which harsh conditions, such as 8 M urea, glass bead lysis using an instrument, and boiling the sample with alkaline extraction etc., are used for yeast protein extraction. Proteins in certain molecular weight range are usually preferentially extracted with these methods. This kit features a single tube protocol, full range of yeast proteins are extracted without bias. The kit works well for both log phase and stationary phase microbes. The whole procedure takes less than 10 min to complete and the protein yield is in the range of 2-5 mg/ml. The materials provided are sufficient for 50 extractions.

Application

Proteins extracted with this kit can be directly used for many downstream applications such as SDS-PAGE analysis, Western blotting, IP, ELISA and enzyme activity assays and proteomic analysis. The buffers are compatible with IMAC resins for his-tagged protein purification

Kit components

1. 20 ml Denaturing Buffer
2. 20 ml Native Buffer
3. 5 g protein extraction powder
4. 4 pestles for 1.5 ml microcentrifuge tube
5. 1.5 ml microfuge tube X 50

Storage: Store the kit at 4C.

Additional Materials Required

Table-Top Microcentrifuge with a maximum rpm of 14,000-16,000.

Important Product Information

The use of protease inhibitors is not necessary prior to extraction. However if downstream application takes significant amounts of time or the protein extract will be stored for longer period of time, addition of protease inhibitors to extraction buffer is recommended. For determination of protein concentration, BCA kit (Pierce) is

recommended. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to the buffer prior to use.

Protocol

1. Harvest microbes of interest by centrifugation in a 1.5 ml microfuge tube. Make sure that the wet volume of pellet is between 20-30 μ l. The volume can be easily estimated by comparing it to a 1.5 ml tube with 30 μ l water.
1. Wash the pellet with one ml water by centrifuging at top speed in a microcentrifuge for 2 min. Remove supernatant completely. Weigh out 80-90 mg protein extraction powder and add to the bottom of the tube (try not to touch the wall of the tube. This can be done by weighing out the powder in a piece of folded wax paper and pour the powder to the bottom of the tube). Add 20 μ l of **denaturing or native buffer** to the bottom of the tube.
2. Grinding the tube repeatedly with the pestle provided for about 2 min with twisting force. Add 150-200 μ l protein extraction buffer used in step 2 above and continue to grind for about thirty seconds (note: The pestle is reusable, for cleaning simply soak it in bleach, rinse with water and dry it with paper towel). Cap the tube and vortex vigorously for 10 seconds.
4. Centrifuge the tube at top speed for 3-4 min. Transfer the supernatant to a fresh tube (this is extracted total protein). If more protein is desired repeat step 3-4 one more time. Typically protein yield is 3-5 mg/ml for denaturing buffer and 2-3 mg/ml for native buffer. Extracted protein can be stored at -80C for future use.

Application tips: The final protein yield is proportional to grinding frequency and time in step 3. The pestles fit the best with 1.5 ml tubes provided.