

Bacterial Protein Extraction Kit

Cat:Bio-PBF-1k

Size: 10xStrength Slotion(1L culuture medium)

Content:

Component	
10x Cell lysis buffer	5 ml
DNase/RNase Solution	200 µl
Lysozyme Solution	1 ml
PMSF Solution	500 µl
SDS gel-loading buffer	0.2 ml

Protocol:

- 1 Collect the protein expressing cells of interest by centrifuging at 5,000 x g for 10 minutes. Remove the media and wash the pellet with 1 x PBS. Re:suspend the cell pellet in 4 ml of 1x cell lysis buffer per 100 ml of cell culture.
- 2 Add 40 µl of the PMSF Solution and 80 µl of the Lysozyme Solution; incubate the cell suspension on ice for 30 minutes.
- 3 Incubate the mixture on a rocking platform for 10 minutes at 4°C.
- 4 Add 20 µl of DNaseI/RNase Solution; continue the incubation by shaking for another 10 minutes at 4°C.
- 5 Remove the insoluble debris by centrifugation at 3,000 x g for 30 minutes at 4°C. Collect the supernatant (cell lysate) in a fresh tube.
- 6 Assay the extraction by analyzing 20 µl of aliquot by electrophoresis through a 10% SDS-PAGE. Store the target protein containing the fraction at -70°C. Alternatively, load the target protein onto the purification column.

Note:

- 1 Perform step 2-5 at 4°C.
- 2 Protease inhibitors may be added. Do not add EDTA or other chelators when use Ni²⁺-affinity column, as the chelators destroy the column's ability to bind histidine.
- 3 It may be necessary to pass the supernatant through a 0.45-µm filter to prevent clogging of the resin during the purification.
- 4 Bacterial cells can be lysed by sonication (3x 10 seconds) for some proteins, however, keep the cells cold (0°C) during sonication.
- 5 It is recommended to use the extracted proteins as soon as possible after extraction.
- 6 When PMSF is dissolved in isopropanol, the solution should be stored at -20°C.

Caution:

Operate with caution while using PMSF. PMSF is extremely toxic and is irritating to the eyes and skin. Wear eye and hand protection to keep the solution away from skin and eyes. This product is for laboratory research use only.

Storage:

Store DNase/ RNase Solution, Lysozyme Solution and PMSF Solution at -20°C. Store other contents at room temperature. For long term storage, keep all contents cold.