



PRODUCT INFORMATION

Bacterial Protein Extraction Kit (Cat# PEB-1000, Cat# PEB-5000)

Production information for PEB-1000 (for bacterial cell cultured in 1 liter medium) and PEB-5000 (for bacterial cell cultured in 5 liter medium)

Content:

Component	PEB-1000	PEB-5000
10x Cell lysis buffer	5 ml	25 ml
DNase/RNase Solution	200 µl	1 ml
Lysozyme Solution	1 ml	5 ml
PMSF Solution	500 µl	2.5 ml
SDS gel-loading buffer	0.2 ml	1 ml

Protocol:

- 1 Collect the cells that express the protein of interest by centrifuging at 5,000 x g for 10 minutes. Remove the media and wash the pellet with 1 x PBS. Resuspend the cell pellet in 4 ml of 1x cell lysis buffer per 100 ml of cell culture.
- 2 Add 40 µl of PMSF Solution and 80 µl of 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 for another 10 minutes at 4°C, and keep shaking.
- 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 aliquot by electrophoresis through a 10% SDS-PAGE. And store the target protein containing fraction at -70°C or directly load onto the purification column.

Note:

- 1 Perform step 2-5 at 4°C.
- 2 Protease inhibitors may be added, but do not add EDTA or other chelator when use Ni²⁺-affinity column, because chelator affects the histidine binding in the column.
- 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 also be lysed by sonication (3x 10 seconds) for isolating some proteins; however, the cells should be on ice during the sonication.
- 5 It is recommended to use the extracted proteins as soon as possible after extraction.
- 6 PMSF is dissolved in isopropanol and should be stored at -20°C.

Caution:

Operate carefully with PMSF, it is extremely toxic and is irritating to eyes and skin. Wear eye and hand protection to keep the solution containing PMSF 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, and store other contents at room temperature. For longer storage, keep all contents cold