Bacterial Protein Extraction Kit

Product information for BS596/BS597:

Introductions:

Bacterial Protein Extraction Kit, is designed for the extraction of biologically active soluble proteins and high purity inclusion bodies from bacterial cells. It is compatible with the FLAG, poly-His and GST fusion protein affinity chromatography purification systems, the kit is also compatible with many enzyme or protein assays and protease inhibitor cocktails. The kit is sufficientt for 1 liter of cell culture (when OD600.=1) or 10x100-200mg wet weight E.coli. Store 10x Extract-EZ B buffer at 4℃; Store DNase I, RNase, Lysozyme and PMSF at -20C. Valid for 1 year.

Components:

Component	BS596	BS597
10x Cell lysis buffer	5 ml	25ml
DNase/RNase	200 ul	1ml
Lysozyme	1 ml	5ml
PMSF	500 ul	2500ul
2xSDS gel-loading buffer	0.2 ml	1ml

Storage Condition:

Transporation at room temperature,after received ,Store DNase/ RNAse, Lysozyme,2xSDS gel-loading buffer and PMSF at –20℃, and store the rest contents at room temperature.

Procedures:

1. Collect the cells that express the protein of interest by centrifuging at 5,000x g for 10 minutes. Re-move the media and wash the pellet with PBS. Resuspend the cell pellet in 4ml of 1x cell lysis buffer per 100ml of cell culture (when OD600.=1).
2. Add 40ul PMSF and 80µl lysozyme, incubate the cell suspension at 37°C for 30 minutes.
3. Incubate the mixture on a rocking platform for 10 minutes.

4. Add 20ul DNaseI/RNase, continue the incubation with rocking for another 10 minutes at 37°C.
5. Remove the insoluble debris by centrifugation at 3,000g for 30 minutes at 4°C. Collect the super-natant (cell lysate) in a fresh tube.
6. Assay the extraction by analyzing 20-µl aliquots by electrophoresis through a 10% SDS-PAGE. and store the fraction containing target protein at -70°C or directly load onto the purification column.

Notes :

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