ab286871 – Bacterial Protein Extraction Reagent

Non-denaturing bacterial proteins and inclusion bodies extraction reagent.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab286871

Storage and Stability

This product may be stored at 4°C for up to 1 year from the date of shipment.

Materials Supplied

Bacterial Protein Extraction Reagent prepared in a 50 mM Tris pH8.0 buffer

Protocol

A. Soluble Protein Extraction:

1) Collect cells by centrifugation at 10,000g x 10 minutes in a pre-weighed centrifuge tube. For a small-scale preparation (e.g., ≤ 1.5 ml), centrifugation can be performed in an eppendorf tube 13000-16000g x 10 min. Remove as much liquid as possible. Determine wet weight of the cell pellet.

2) Resuspend the pellet in minimum 4 ml of the Bacterial Protein Extraction reagent (prewarmed to room temperature) per 1g of wet cell paste by pipetting and/or gentle vortexing.

Optional:

a) Addition of protease inhibitors is recommended at this time.

b) Addition of Turbonuclease (10-100 U/g wet cell paste) and Lysozyme (5 KU/g wet cell paste) may reduce viscosity and improve protein extraction.

3) Incubate the cell suspension by shaking or slow mixing for 5 minutes or longer.

4) Remove insoluble debris by centrifugation at 16,000g x 20 min at 4°C. The supernatant containing the soluble proteins can now be analyzed or further purified such as using a Ni-NTA column or stored at -20°C for future use.

∆ **Note**: The pellet contains inclusion bodies. If desired, save the pellet for inclusion body purification.

B. Inclusion Body Purification:

1) Resuspend the pellet (from Step A4) in the same volume of Bacterial Protein Extraction reagent which was used to resuspend the cell pellet (Step A2) by pipetting up and down and vortexing to obtain an even suspension. The pellet needs to be completely resuspended.

2) Add lysozyme to a final concentration of 1 KU/ml, mix gently and incubate at room temperature for 10 min.

3) Centrifuge at 5000g x 15 min. at room temperature. Carefully remove supernatant.

4) Resuspend the pellet with 1:10 diluted Bacterial Protein Extraction using about 8 ml per gram of original wet cell pellet and mix by vortex; centrifuge at 5000g x 15 min. at room temperature to collect the inclusion body.

5) Repeat the step 4 (above) two more times.

6) Take aliquot for SDS-PAGE analysis. Pellet the inclusion bodies at 16000g x 15 min at 4 °C. Remove supernatant. The purified inclusion bodies can be used immediately or stored at -80 °C for future use.

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Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)