

Minute™ Bacterial Total Protein Extraction Kit

Catalog number: SB-004

Description

Invent Biotechnologies Minute™ bacterial total protein extraction kit is composed of optimized denaturing cell lysis buffers and protein extraction filter cartridges with 2.0 ml collection tubes. The kit is designed to rapidly extract denatured proteins from bacteria. The kit contains sufficient materials for extraction of total proteins from 100 ml *E.coli* culture.

Application

Minute™ bacterial total protein extraction kit is designed to rapidly extract total proteins from cultured bacterial cells for applications such as SDS-PAGE, immunoblottings, ELISA and other applications. This kit provides the most rapid method for preparation of whole bacterial cell extract.

Buffer Formulation: Proprietary

Kit Components

1. 25 ml bacterial lysis buffer A (Part#: SB-004-A)
2. 2.5 ml buffer B (Part#: SB-004-B)
3. 50 protein extraction filter cartridges (Part#: P-001)
4. 50 collection tubes with cap (Part#: P-002)

Storage

Store at room temperature.

Important Product Information

The Minute™ bacterial total protein extraction kit is designed to extract total protein rapidly from bacteria. 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 inhibitor to extracted lysate is recommended. For determination of protein concentration BCA kit (Pierce) is recommended. The protein extraction filter cartridge has the capacity of 500 µl. Multiple filter cartridges may be used if larger amount of cell lysate is desired. To study protein phosphorylation, phosphatase inhibitors must be added to lysis buffer prior to use.

Additional Materials Required

1 X PBS

Vortexer

Table-Top Microcentrifuge

BCA Protein Assay Kit (Pierce, Cat # :23227)

Protein Extraction Procedures

1. Prior to protein extraction pre-chill the protein extraction filter cartridge with collection tube on ice.
2. Pellet cells from bacterial culture in a 1.5-2.0 ml microcentrifuge tube with a table-top microcentrifuge at top speed for 1-2 min. Remove the supernatant and wash the cell pellets in cold PBS once. Aspirate the supernatant and leave small amount of PBS (about equivalent volume of packed cells) in the tube. Vortex briefly to resuspend the cells.
3. Add appropriate amounts of room temperature bacterial lysis buffer A to cell suspension (Table 1). Vortex vigorously for 10 second.

Note: presence of small amount of unlysed cells would not affect the quality of the samples.

4. Add appropriate amounts of buffer B (1/10 of buffer A) to the lysate and vortex vigorously for 10 seconds.
5. Transfer/pour the cell lysate to pre-chilled filter cartridge, and centrifuge in a microcentrifuge at 14,000-16,000 rpm for 30 seconds.
6. The clear cell lysate in collection tube is ready to use. Discard the filter cartridge according to your institution's waste disposal protocol. The protein yield with this method is about 2.0-3.0 mg/ml..

Table 1, Lysis Buffer Volume for Different Packed Cell Volume*

Packed cell volume (µl)	Lysis buffer A (µl)	Buffer B (µl)
5	50	5
10	150	15
20	250	25
40	500	50

*1.5 ml overnight *E.coli* culture is equivalent to about 10 µl packed cells

Troubleshooting

Problem	Solution
Precipitates found in bacterial lysis buffer A	Warm to room temperature

Precipitates in protein extract on ice	Warm to room temperature prior to use
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Appendix: Additional product information

Fig. 1, SDS-PAGE of extracted proteins. Lane 1, total proteins isolated from *E. coli* by repeated sonication. Lane 2 and 3, represent two separated experiments using Minute™ Bacterial Total Protein Extraction Kit.

