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A Geno Technology, Inc. (USA) brand name

FOCUS™ Bacterial Proteome

(Cat. #786-258)

INTRODUCTION

FOCUS™ Bacterial Proteome is specifically designed for bacterial research and supplied with bacterial specific reagents, which extracts and solubilizes nearly all of the proteins from E. coli, including membrane as well as soluble proteins. Extraction is based on gentle lysis of bacterial cells with Longlife™ Lysozyme™ enzyme specifically prepared for bacterial lysis and stabilized for long term use. This kit is provided with an optional protocol for the formation of spheroplast and removal of lytic enzyme (Lysozyme) prior to lysis and extraction of bacterial proteins. It is supplied with a proprietary and strong chaotropic extraction buffer to solubilize even the most difficult proteins. It is suitable for IEF & 2D gel electrophoresis.

ITEM(S) INCLUDED (Cat. #786-258)

Description	Size
Bacterial Suspension Buffer	25ml
Longlife™ Lysozyme™ (1,500 units/µl)	1ml
FOCUS™ Protein Solubilization Buffer [FPS Buffer]	25g
DILUENT- III	30ml

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival store the kit components as individually marked. Stable for 1 year when stored and used as recommended.

ADDITIONAL ITEM(S) REQUIRED

Sonicator, centrifuge, centrifuge tubes, glass beads, reducing agent, carrier ampholyte and protease inhibitor cocktail.

PREPARATION BEFORE USE

The kit is supplied with FPS Buffer. Allow the FPS Buffer to warm to room temperature before opening the bottle. Read the instructions on the bottles carefully before use. Just before use, hydrate an appropriate amount of the FPS Buffer with DILUENT-III. Add needed agents such as reducing agent, carrier ampholyte, and if necessary, an appropriate amount of protease cocktail (ProteaseArrest™, Cat# 786-108).

PROTOCOLS

A. Longlife™ Lysozyme™ Assisted Extraction

1. Suspend bacterial cell pellet in 5-10 times volume of Bacterial Suspension Buffer.
2. Vortex until the cell suspension becomes homogenous.
3. Flick the vial containing *Longlife*™ Lysozyme™ to mix the suspension. Add 10µl of Lysozyme per 100µl of bacterial cell pellet. Gently mix the content.
4. Incubate the cell suspension at 37°C for 30-60 minutes.
5. At the end of incubation, centrifuge the suspension at 5,000x g for 10 minutes. Remove and discard the supernatant carefully, leaving the spheroplast pellet in the tube.

   OPTIONAL: *Re-suspend the spheroplast pellet in 5-10 volume of the Bacterial Suspension Buffer. Centrifuge again as above and discard the supernatant.*
6. Suspend the spheroplast pellet in freshly prepared FPS Buffer, 5-10 times the total volume of the spheroplast pellet.
7. Sonicate the suspension with an ultrasonic probe to break down the cell membrane and genomic DNA. Sonication should be performed in an ice cold bath and during sonication; care must be taken to prevent heating. Sonication should be performed with bursts of 20-30 seconds and chill the suspension between ultrasonic bursts.
8. Centrifuge at 20,000x g for 30 minutes at 20°C and collect clear lysate.

   OPTIONAL*: Suspend the residual cell debris in 1/4 the volume of FPS Buffer used in the previous step. Sonicate the suspension once briefly. Collect the extract and pool with the first extract supernatant. Store bacterial protein extract at -70°C until used.*

B. Sonication & Glass Beads Assisted Extraction

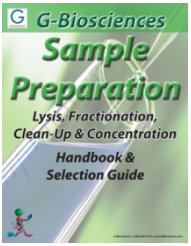
1. Suspend bacteria cell pellet in freshly prepared FPS Buffer- use 5-10 times the size of pellet. Add 5 times volume of glass beads (0.1 mm diameter). Sonicate the suspension with an ultrasonic probe to break the cells and break down the genomic DNA. Sonication should be performed in an ice cold bath and during sonication; care must be taken to prevent heating. Sonication should be performed with bursts of 20-30 seconds and chill the suspension between ultrasonic bursts.
2. It may take 10 - 20 minutes depending on the sample size and the strength of sonicator. Other mechanical devices and grinders may be used instead of an ultrasonic probe.
3. Centrifuge the homogenate at 20,000 x g for 30 minutes at 20°C to pellet the debris and collect the clear lysate.

   OPTIONAL: *Suspend the residual cell debris in 1/4 the volume of FPS Buffer used in the previous Step 1. Sonicate the suspension once briefly. Repeat step 2. Collect the extract and pool with the first extract supernatant. Store the bacterial protein extract at -70°C until used.*
4. Determine protein concentration (use Non-Interfering Protein Assay, *G-Biosciences* Cat# 786-005). Make an appropriate dilution in FPS Buffer before running IEF/2D gels.

   NOTE: *Proteins solubilized in FPS Buffer that contains CHAPS may not be suitable for running SDS-PAGE. Use PAGE Perfect (G-Biosciences Cat # 786-123) to remove detergent before running SDS-PAGE electrophoresis.*

RELATED PRODUCTS

Download our Sample Preparation Handbook.



http://info.gbiosciences.com/complete-protein-sample-preparation-handbook

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