Protocol

Creation Date: 7/18/2014
Revision Date: 1/24/2019

Bacterial Cell Lysis Kit

GoldBio Cat# GB-177 & GB-176

Introduction

The *Bacterial Cell Lysis Kit* has been developed for the extraction of soluble proteins and inclusion bodies from bacterial cells. It is a proprietary improvement on the lysozyme based lysis, which allows extraction of soluble proteins and concurrent removal of nucleic acids (DNA & RNA) released during cell lysis. The lysis eliminates viscosity build-up, allowing effective clarification with lower centrifugal force. 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 the bacterial proteins.

Bacterial Cell Lysis Buffer is based on organic buffering agents and utilizes a mild non-ionic detergent and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. Depending on the application, additional agents such as reducing agents, chelating agent, and protease inhibitors may be added into *Bacterial Cell Lysis Buffer*. This reagent has been tested for use with several widely used bacteria including *E. coli* strains. *Bacterial Cell Lysis Buffer* eliminates the need for laborious mechanical lysis of bacterial cells and removal of DNA/RNA with nuclease treatments. The proprietary combination of this reagent provides a simple and versatile method of bacterial protein extraction and isolation of inclusion bodies. *Bacterial Cell Lysis Buffer* is suitable for preparation of spheroplasts, lysis and extraction of proteins from bacterial cells and isolation of inclusion bodies. Bacterial Cell Lysis Buffer is compatible with most downstream applications including running various chromatography, gel electrophoresis applications, and protein folding procedures.

Storage and Handling

The kit is shipped at ambient temperature. Upon arrival store the kit components at 4°C except Lysozyme which is stored at -20°C. Stable for 1 year when stored and used as recommended.

Materials

- Centrifuge
- Test tubes
- Incubator
- Additional volume of the Bacterial Lysis Buffer may be purchased separately for downstream applications such as chromatography, dialysis, etc.

Bacterial Cell Lysis Kit (Cat# GB-176)

Gold Biotechnology
St. Louis, MO
Ph: (800) 248-7609
Web: www.goldbio.com
Email: contactgoldbio86@goldbio.com

- Bacterial Cell Lysis Buffer
- Bacterial Suspension Buffer
- Lysozyme (with nucleases)

Bacterial Cell Lysis Buffer (Cat# GB-177)

- Bacterial Cell Lysis Buffer

Method

Depending on applications, DTT (GoldBio Cat# DTT10) and EDTA (GoldBio Cat# E-210) may be added. Prepare an appropriate volume of the Bacterial Cell Lysis Buffer use by adding DTT and EDTA both to a final concentration of 5mM. If the presence of a divalent metal ion is necessary for any application, do not add EDTA; instead, add an appropriate divalent salt to a final concentration of 5mM.

The lysozyme contains 40 mg/ml lysozyme (~80 kU) supplemented with 800 U/ml DNase and 24 U/ml RNase. We recommend using the lysozyme at a final concentration of 0.1-1 mg/ml. Higher concentrations of lysozyme will not improve lysis efficiency and may have an inhibitory effect.

If the inhibition of protease activity is required, add a cocktail of protease inhibitors to prevent protease activities during the extraction procedure (see Related Products for protease inhibitor cocktails).

Protein extraction with concurrent removal of nucleic acids

1. Pellet bacterial cells (bacterial culture, OD600 1.5-3.0) by centrifugation at 5000 x g for 10 minutes. Suspend the cell pellet in 5-10x volumes of the Bacterial Lysis Buffer (cell pellet size 25 µl use 125-250 µl Bacterial Cell Lysis Buffer).

2. Gently pipet up and down until the cell suspension is homogeneous. Incubate the suspension for 5 minutes in ice. Gently pipet again to suspend the cells.

3. Vortex the tube containing Lysozyme to mix the frozen suspension. Add 5 µl Lysozyme for each 100 µl cell suspension in Bacterial Cell Lysis Buffer. Gently mix the content.

4. Incubate the suspension at 37°C for 30-60 minutes.

   Note: (OPTIONAL). Lysis can be monitored by taking 25 µl of suspension and mixing with 1 ml Bacterial Cell Lysis Buffer and reading the optical density at OD 590 nm.

5. At the end of incubation period, vortex the content of the tube several times (30 seconds each) to complete the lysis. Lysis may be further assisted by pipetting the

suspension up and down a few times with a narrow bore pipet tip or a 20-gauge syringe needle.

Note: Additional volumes of the Bacterial Cell Lysis Buffer may be purchased separately for downstream applications such as chromatography, dialysis, etc.

6. Removing Nucleic Acids**.** During lysis, cellular DNA and RNA are cleaved which reduces the viscosity of the lysate. Some DNA fragments may survive, which would not interfere with downstream processing. However, for complete removal of nucleic acids, do not add EDTA in to the Bacterial Cell Lysis Buffer. After lysis is complete EDTA may be added to a final concentration of 2.5mM.

7. Centrifuge the lysate at 20,000 x g, 4°C for 30 minutes and collect the clear lysate.

Note*:* See Isolation of inclusion bodies section.

Lysate is now ready for any application, including biological activity assays, electrophoresis, protein purification, or further analysis.

Note: Titer Plate Applications. For high throughput titer plate applications the protocol can be modified by proportionately reducing the volumes.

Protein extraction with spheroplast formation
Suitable when Lysozyme contamination is not acceptable.

1. Pellet bacterial cells (bacterial culture, OD600 1.5-3.0) by centrifugation at 200-500 x g for 10 minutes. Suspend the cell pellet in 5-10 volumes of the Bacterial Suspension Buffer (cell pellet size 25 µl use 125-250 µl Bacterial Suspension Buffer).

2. Gently pipet up and down until the cell suspension is homogeneous. Incubate the suspension for 5 minutes in ice. Gently pipet again to suspend the cells.

3. Vortex the tube containing Lysozyme to mix the frozen suspension. Add 5 µl Lysozyme for each 100 µl cell suspension in Bacterial Suspension Buffer. Gently mix the content.

4. Incubate the suspension at 37°C for 30-60 minutes.

Note: (OPTIONAL). Lysis can be monitored by taking 25 µl suspension and mixing with 1 ml Bacterial Cell Lysis Buffer and reading the optical density at OD 590 nm.

5. At the end of incubation, centrifuge the suspension at 5,000 x g for 10 minutes. Remove and discard the supernatant carefully, leaving the spheroplast pellet in the tube.

Note: (OPTIONAL). Re-suspend the spheroplast pellet in 5-10 volumes of the Bacterial Suspension Buffer. Centrifuge again as above and discard the supernatant.

6. Lysis. For lysis, suspend the spheroplast pellet in an appropriate volume of the Bacterial Cell Lysis Buffer (2-3 times the volume of spheroplast pellet). Pipet the suspension up and down a few times. Vortex periodically and incubate on ice for 30 minutes. The lysis may be further facilitated by incubating the cells for 1-3 minutes at 37°C or a brief sonication step. Please note, the higher Bacterial Cell Lysis Buffer to spheroplast pellet ratio the better the cells will lyse.

7. Centrifuge the lysate at 20,000 x g, for 30 minutes at 4°C and collect the clear lysate.

Note: See Isolation of inclusion bodies section.

Lysate is now ready for any application, including biological activity assays, electrophoresis, protein purification, or further analysis.

Isolation of Inclusion Bodies

1. For inclusion bodies isolation, after the lysis step centrifuge the bacterial lysate at 30,000 x g for 30 minutes at 4°C.

2. Collect the inclusion bodies pellet and wash twice with 10 fold diluted Bacterial Cell Lysis Buffer (e.g., suspend in buffer and centrifuge to pellet the inclusion bodies).

3. Collect the inclusion bodies for solubilization and re-folding.

Associated Products

1. **ProBlock™ Gold (Cat # GB-108):** A cocktail of protease inhibition for use during protein extraction and purification. ProBlock Gold™ inhibits a broad spectrum of serine, cysteine and metalloproteases as well as calpains.

2. **ProBlock™ Gold Bacterial (Cat # GB-330):** A cocktail of protease inhibition for use during protein extraction and purification from bacterial cells. ProBlock Gold™ inhibits a broad spectrum of bacterial serine, cysteine and metalloproteases as well as aspartic proteases and aminopeptidases.

3. **ProBlock™ Gold Bacterial 2D (Cat # GB-376):** A cocktail of protease inhibition for use during recombinant protein extraction and purification from bacterial cells. ProBlock Gold™ inhibits a broad spectrum of bacterial serine, cysteine and metalloproteases as well as aspartic proteases and aminopeptidases.