Protocol

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Dr. Gold's Bacterial Protein Extraction Protocol
For the Extraction of Expressed Proteins

Introduction

Dr. Gold's Bacterial Protein Extraction Reagent is a powerful, next-generation solution designed for efficient extraction of expressed proteins from bacterial cells—now reengineered for improved performance and cost-effectiveness. Built on the foundation of our trusted Bacterial Cell Lysis Buffer, this ready-to-use formulation offers enhanced yield and reproducibility without the need for harsh mechanical disruption.

The reagent includes lysozyme, nucleases, and a specially optimized salt solution to promote full membrane disruption and maximize the recovery of soluble proteins.

> **Note: The Salt Solution is not strictly required for lysis, but our research has shown they significantly enhance protein solubility and yield.**

Materials

- Dr. Gold's Bacterial Protein Extraction Reagent
- GB Enzyme Mix
- Salt Solution
- Pipet
- Centrifuge tubes
- Microcentrifuge tubes
- Centrifuge
- Shaker incubator
- Incubator (22°C)
- UV/Vis Spectrophotometer

Storage and Handling

- This product may be shipped with blue ice. Dr. Gold's Bacterial Protein Extraction Reagent and Salt Solution should be stored at 4°C, GB Enzyme Mix should be stored at -20°C immediately upon arrival. When stored under the recommended conditions and handled correctly, these products should be stable for at least 1 year from the date of receipt.
- Thaw the **GB Enzyme Mixture** on ice. Gently flick the tube to resuspend any settled material.

Method

Note: This protocol assumes you have a culture of *E. coli* cells expressing your protein of interest. The culture should be properly induced and grown under optimized conditions. For example, *E. coli* cells carrying a plasmid with an IPTG-inducible promoter (e.g., lac) may be induced with 0.1M IPTG and grown overnight at 30°C.

1. **Measure cell density**
   Measure the optical density at 600 nm (A_{600}) of the *E. coli* culture. You will need this to determine the amount of required lysis buffer on step 3.

2. **Harvest the cells**
   Centrifuge the culture at **3,500 × g for 15 minutes** (or 10,000 × g for 5 minutes) to pellet the cells. *Note:* 15 minutes at 3,500 × g is generally ideal if your centrifuge rotor has a 12 cm path length.

3. **Resuspend in lysis buffer**
   Discard the supernatant and gently resuspend the pellet in **pre-warmed (22°C) lysis buffer**. If desired, you may add protease inhibitors to the lysis buffer prior to resuspending.

   > **Note: For Protease inhibitors, we recommend using ProBlock™ Gold Bacterial Protease Inhibitor Cocktail (GoldBio Catalog # GB-330)**

   Use **1.3 mL of lysis buffer per 10 mL of culture at A_{600} = 2.0**. Adjust proportionally for different cell densities and volumes.

   ***Important:* Do NOT vortex. Resuspend by gentle pipetting or swirling.**

4. **Add enzyme mixture**
   Thaw the **GB Enzyme Mixture** on ice. Gently flick the tube to resuspend any settled material.

   Add **1/100 volume** of enzyme mix to the resuspended cells. Mix gently.

5. **Incubate for enzymatic lysis**
   Incubate at **22°C for 30 minutes**, gently agitating every 10 minutes.

   ***Observation:* Some debris may settle as the enzymes digest cell walls, DNA, and RNA.**

6. **Add salt solution**
   Add **1/10 volume** of the included **Salt Solution** to the lysate.

   The solution should become more translucent, though some opaqueness may remain. Place the mixture on **ice** for 5-10 minutes.

   > **Note: If analyzing lysate via SDS-Page, remove a 0.5 mL aliquot into a microcentrifuge tube and process separately for soluble/insoluble fraction analysis (See instructions for Optional Analysis via SDS-Page below).**

7. **Clarify lysate**
   Centrifuge the mixture at **10,000 × g for 10 minutes at 4°C** to pellet cell debris.

8. **Harvest supernatant**
   Carefully transfer the **clarified supernatant** (soluble protein fraction) for downstream analysis or purification.

Analysis via SDS-PAGE (Optional):

1. For the **lysate (supernatant):** After centrifugation according to **Step 8**, remove supernatant and mix directly with SDS-Page loading buffer for evaluation.

2. For the **pellet (insoluble fraction):** Resuspend pellet in **50 µl of SDS-Page loading buffer**, heat at **100°C for 3 minutes**, then centrifuge at **10,000 × g for 5 minutes**.

3. Analyze both supernatants by SDS-PAGE to evaluate soluble vs. insoluble protein.

Associated Products

- Dr. Gold's Bacterial Protein Extraction Reagent (GoldBio Catalog # GB-500)
- ProBlock™ Gold Bacterial Protease Inhibitor Cocktail (GoldBio Catalog # GB-330)