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Cell Lysis Reagent

Product Insert

Product # 18800 (100 mL)
Product # 18801 (500 mL)

Norgen's Cell Lysis Reagent is designed for the gentle lysis of bacterial cells, in order to assist in the extraction of inclusion body proteins. Cell lysis is accomplished through non-ionic detergent chemical disruption in conjunction with mechanical disruption. The use of a needle and syringe during the procedure helps to reduce viscosity and facilitate purification, producing proteins that are often greater than 95% pure. It should be noted that Norgen's Cell Lysis Reagent does not solubilize inclusion bodies.

Product Components

Component	Product # 18800	Product # 18801
Cell Lysis Reagent	100 mL	500 mL
Product Insert	1	1

Storage Conditions and Product Stability

The Cell Lysis Reagent should be stored at 4°C. This reagent should remain stable for at least 1 year in its unopened container.

Precautions and Disclaimers

This reagent is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

Customer-Supplied Reagents and Equipment

- Centrifuge
- Appropriate centrifuge tubes
- Lysozyme
- Sterile deionized or Milli-Q® water
- 18 gauge needle and 10 mL syringe (large scale extraction)
- 20 gauge needle and 1 mL syringe (small scale extraction)

Procedure

Notes prior to use:

- Ensure that no precipitates have formed. If necessary, warm and mix well until the solutions become clear again.
- Prepare an appropriate amount of lysozyme solution for the desired number of preparations, using Table 1 below.
- Cell Lysis Reagent does not solubilize inclusion bodies.
- The efficiency of inclusion body extraction will vary depending on the bacterial strain being used.
- Growth and induction conditions are dependent upon the host strain and gene expression vector utilized.
- To ensure the option of scaling-up clones that are found to contain the protein of interest it is recommended that the user preserve stocks of uninduced bacteria for each clone tested.

Table 1: Lysozyme concentrations for Gram-negative and Gram-positive strains.

Bacteria Type	Lysozyme Concentration in Sterile Water
Gram-Negative	0.4 mg/mL
Gram-Positive	3 mg/mL

Procedures

A. Small Scale Extraction (From 1.5 mL of Culture)

The small scale extraction procedure is designed to rapidly screen bacterial clones for expression of recombinant proteins in inclusion bodies. Test tube cultures containing 2 mL of culture medium are normally initiated by inoculation with single colonies picked from culture plates. All centrifugation steps are carried out at 14,000 x g in a benchtop microcentrifuge. Please check your microcentrifuge specifications to ensure proper speed. All centrifugation steps are performed at room temperature. Centrifugation at 4˚C will not adversely affect performance.

1. At the end of the induction period, transfer 1.5 mL of the bacterial culture into a microcentrifuge tube.

2. Centrifuge for one minute and discard supernatant.

3. Freeze pellet at -20°C or lower using a freezer or liquid N_2.

   Note: This can be a convenient place to stop the protocol overnight. The procedure can then be carried on from this point the next day.

4. Thaw pellet at room temperature or 37°C.

5. Resuspend pellet in 200 µL of **Lysozyme** solution (see Table 1 above) using pipette and vortex.

6. Incubate the suspension at room temperature for 10 minutes.

7. Add 200 µL of Cell Lysis Reagent to the suspension.

8. Assemble a 20 Gauge needle with a 1 mL syringe (not provided). Carefully disrupt the bacterial pellet by drawing it along with the Cell Lysis Reagent through the needle and ejecting the suspension back into the microcentrifuge tube. Pass through the needle 15 to 20 times. This same needle and syringe may be used for all subsequent steps.

9. Centrifuge the suspension for 10 minutes and carefully discard the supernatant.

   Important! This supernatant may be quite viscous. Do not disturb the pelleted material when discarding the supernatant. (The supernatant may be saved in a fresh microcentrifuge tube for comparative analysis of the soluble proteins present in this fraction.)

10. Using the needle-and-syringe technique described in step 6, add 200 µL of **Cell Lysis Reagent** to the tube and carefully resuspend the pellet. A few passes through the needle is sufficient to prepare a homogenous suspension.

11. Prepare a 10-fold dilution of **Cell Lysis Reagent** (mix one part of the stock Cell Lysis Reagent with nine parts of sterile deionized or Milli-Q® water). Add 600 µL of this dilution to the suspension prepared in step 10 and pass through the needle a few times.

12. Centrifuge for 10 minutes and discard the supernatant.

    Important! Use caution to avoid accidental removal of pelleted material.

13. Add 800 µL of the **diluted Cell Lysis Regent** to the pellet and resuspend using the needle and syringe until homogenous.

14. Centrifuge for 10 minutes and discard supernatant.

15. Ensure that the pellet is relatively dry by tapping out residual liquid or by careful use of aspiration.

16. Resuspend inclusion body pellet in a buffer of choice.

Note: Inclusion bodies can be efficiently solubilized using Norgen's **Inclusion Body Solubilization Reagent** (Part Number 18700).

B. Large Scale Extraction (From 100 mL of Culture)

1. At the end of the induction period, transfer 100 mL of the bacterial culture to a 250 mL centrifuge bottle.

2. Centrifuge for five minutes at 15,000 x g and discard supernatant.

   Note: Use of a 250 mL centrifuge bottle may be eliminated by initially spinning the bacterial culture in a 40 mL centrifuge tube. This would require three spins of five minutes each at 15,000 x g to accommodate the 100 mL of culture.

3. Freeze pellet at -20^{o}C or lower using a freezer or liquid N_2.

   Note: This can be a convenient place to stop the protocol overnight. The procedure can then be carried on from this point the next day.

4. Thaw pellet at room temperature or 37^{o}C.

5. Resuspend the pellet in 10 mL of **Lysozyme** solution (see Table 1 above) using a vortex.

6. Incubate for 10 minutes at room temperature.

7. Add 15 mL of **Cell Lysis Reagent** to the suspension.

8. Assemble an 18 gauge needle with a 10 mL syringe (not provided). Carefully disrupt the bacterial pellet by drawing it along with the **Cell Lysis Reagent** through the needle and ejecting the suspension back into the centrifuge bottle. Pass through the needle 10 to 15 times.

9. Transfer resuspension into a 40 mL centrifuge tube and centrifuge for 5 minutes at 27,000 x g. Carefully discard supernatant.

   Important! This supernatant may be quite viscous. Do not disturb the pelleted material when discarding the supernatant. (The supernatant may be saved in a fresh 15 mL conical tube for comparative analysis of the soluble proteins present in this fraction.)

10. Prepare a 10-fold dilution of the **Cell Lysis Reagent** (mix one part of the stock **Cell Lysis Reagent** to nine parts of sterile deionized or Milli-Q® water).

11. Using the needle-and-syringe technique described in step 6, add 5 mL of **Cell Lysis Reagent** and 10 mL of the **diluted Cell Lysis Reagent** to the tube and carefully resuspend the pellet. Approximately 10 passes through the needle should be sufficient to prepare a homogeneous suspension. You can use the same needle and syringe from step 6.

12. Centrifuge for 5 minutes at 27,000 x g and discard supernatant.

    Important! Use caution to avoid accidental removal of pelleted material.

13. Add 15 mL of the **diluted Cell Lysis Reagent** to the pellet and resuspend using the needle and syringe until homogeneous.

14. Centrifuge for 5 minutes at 27,000 x g and discard supernatant.

15. Ensure that the pellet is relatively dry by tapping out residual liquid or by careful use of aspiration.

16. Suspend inclusion body pellet in a buffer of choice.

Note: Inclusion bodies can be efficiently solubilized using Norgen's **Inclusion Body Solubilization Reagent** (Part Number 18700).

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
No inclusion body pellet is observed	Improper induction of gene expression.	Consult the manufacturer's expression system literature.
	Gene product does not produce inclusion bodies.	Reassess the expression cassette.
Inefficient cell lysis.	Freeze / thaw step was not performed.	The freeze / thaw step is known to increase lysis efficiency. Repeat the protocol using the recommended freeze / thaw conditions.
	Lysozyme may be required to increase lysis efficiency.	Add lysozyme to concentrations recommended by supplier.
	Mechanical disruption of cells was inefficient.	Increase the number of passages through the needle and syringe.
Supernatant following first spin of cell lysis is too viscious.	Liberation of nucleic acids following lysis.	Increase the degree of mechanical disruption by passing bacteria / lysis reagent through the needle at least five more times. Alternatively, add an appropriate amount of DNaseI.

Related Products	Product #
Inclusion Body Solubilization Reagent	18700
Inclusion Body Micro Kit	10300
Inclusion Body Maxi Kit	17700
ProteoLadder 100	12300

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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