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Instruction Manual

Relay™ 96 Protein Screen

For high-throughput isolation of recombinant proteins from bacterial cells

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General Information

Introduction

The Relay™ 96 Protein Screen provides a simple and rapid high-throughput method for isolating soluble and insoluble fractions of the recombinant proteins from various types of cell cultures. The protocol eliminates the need to harvest cells and does not require any special equipment.

The Relay™ 96 Protein Screen consists of a 96-well Filter Plate containing a solid phase lysis matrix. To isolate your recombinant protein from a cell culture, add the cell culture directly to the Filter Plate, add lysis buffer, and centrifuge the plate.

After centrifugation, the soluble protein is collected into the Receiver Plate. The cell debris, chromosomal DNA, and insoluble proteins remain trapped in the Filter Plate. The insoluble proteins can be isolated from the same sample using the Denaturing Buffer.

Contents

The components included in Relay™ 96 Protein Screen are listed below. Sufficient reagents are provided in the kit to perform 384 isolations.

Item	Amount	Catalog no.
Relay™ 96 Protein Screen System	Lysis Buffer – 25 ml Denaturing Buffer – 80 ml 96-Well Filter Plates – 4 96-Well Receiver Plates – 8	12346-011
Relay™ 96 Protein Screen Filter Plates	25	12346-045
Relay™ 96 Protein Screen Lysis Buffer	150 ml	12346-029
Relay™ 96 Protein Screen Denaturing Buffer	500 ml	12346-037
Relay™ 96 Protein Screen Receiver Plates	50	12346-052

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General Information, Continued

Shipping and Storage

All components of the Relay™ 96 Protein Screen are shipped at **+4°C**. Upon receipt, store the denaturing buffer at **+4°C** and all the other components at room temperature.

Advantages of Relay™ 96 Protein Screen

Using the Relay™ 96 Protein Screen to isolate your recombinant proteins from cell cultures has the following advantages:

- Rapid, inexpensive method designed for high-throughput application
- Gentle detergent lysis method ensures minimal protein denaturation
- Designed to isolate soluble and insoluble proteins from the same sample in 30 minutes
- Minimal contamination of the protein sample with chromosomal DNA
- Compatible with difficult mucoid strains such as BL21
- Protein sample suitable for downstream applications such as enzyme assays, ELISA, and affinity chromatography

Materials Supplied by the User

- Cell culture containing your recombinant protein
- Centrifuge capable of centrifuging 96-well plates
- For bacterial cells, Lysozyme
- Gels for analyzing protein samples. We recommend using NuPAGE® Novex 10% BisTris Gels (Cat. no. NP0301).
- For proteins with low expression levels from insect cells, 50 mM phosphate and 100 mM NaCl to use as a buffer solution (see page 7).

Product Qualification

Protocols, plates, and buffers were tested using BL21 (DE3) *E. coli*. Presence of isolated proteins was confirmed on NuPAGE® Novex 10% BisTris Gels against Mark12™ Standards.

Bacterial Cells

Introduction

This section provides protocols for isolating soluble and insoluble proteins from bacterial cells.

Preparing Lysis Buffer with Lysozyme

To lyse bacterial cells, you must add Lysozyme to the Lysis Buffer supplied in the kit.

Prepare 6 ml of Lysis Buffer containing Lysozyme in a final concentration of 25 µg/ml as described below for use with one 96-well plate. If you are using more than one 96-well plate, scale up accordingly.

To prepare the appropriate concentration of Lysozyme in Lysis Buffer:

1. Dissolve 25 mg of Lysozyme in 1 ml sterile water
2. Add 6 µl of this solution to 6 ml Lysis Buffer.
3. Mix well to achieve a final concentration of 25 µg/ml.
4. Proceed to the following protocol.

Continued on next page

Bacterial Cells, Continued

Isolating Proteins from Bacterial Cells

Use the following protocol to recover soluble and insoluble proteins from bacterial cells. Before proceeding, prepare the Lysis Buffer with Lysozyme as described on the previous page.

Do not place the Filter Plate on any absorbent surface during the protocol to prevent the loss of sample

1. If you are using bacterial cells from a growth block, mix cultures by pipetting up and down several times.
2. Place the Filter Plate on top of a Receiver Plate. Transfer 150 µl of induced cell culture to each well of the Filter Plate.
3. Add 50 µl of Lysis Buffer containing Lysozyme to each well of the Filter Plate. All of the Lysis Buffer may not absorb into the filter. Do not mix by pipetting.
4. Incubate the stacked plates at room temperature for 10–15 minutes.
5. Centrifuge the stacked plates at 1000–2500 × *g* for 5 minutes at room temperature. You can centrifuge at +4° C if your protein is sensitive to high temperature.
6. Soluble proteins will be in the Receiver Plate. Remove the Filter Plate and set aside. (Do not place it on an absorbent surface if you want to recover the insoluble proteins.) Analyze 15 µl of the soluble proteins by SDS-PAGE.

After you have isolated the soluble proteins, insoluble proteins will remain in the Filter Plate. Use the following procedure to recover these proteins.

7. Place the Filter Plate containing your insoluble protein on top of a new Receiver Plate.
8. Add 200 µl of Denaturing Buffer to each well of the Filter Plate. All of the Denaturing Buffer may not absorb into the filter.
9. Incubate the stacked plates at room temperature for 10 minutes.
10. Centrifuge the stacked plates at 1000–2500 × *g* for 5 minutes at room temperature. You may centrifuge the plates at +4°C if your protein is sensitive to high temperature.
11. Insoluble proteins will be in the Receiver Plate. Discard the Filter Plate. Analyze 15 µl of the insoluble proteins by SDS-PAGE.

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Isolating Proteins with Low Expression Levels from Bacterial Cells

The following protocol can be used to isolate proteins with low expression levels from bacterial cells.

Before proceeding, prepare the Lysis Buffer with Lysozyme as described on page 3.

Note: Do not place the Filter Plate on any absorbent surface during the protocol to prevent the loss of sample.

1. Centrifuge the cell culture at 3000 × *g* or higher for 10 minutes to pellet the cells.
2. Remove some of the media to achieve the desired concentration. You can concentrate the cells up to OD_{600} = 15 (higher concentrations may clog the filter).
3. Resuspend the cells in the media by vortexing briefly or pipetting up and down.
4. Place the Filter Plate on top of a Receiver Plate. Add 150 µl of cell culture to each well of the Filter Plate.
5. Add 50 µl of Lysis Buffer (with Lysozyme) to each well of the Filter Plate and incubate for 10–15 minutes at room temperature. All of the Lysis Buffer may not be absorbed into the filter. Do not mix by pipetting.
6. Centrifuge the stacked plates at 2000 × *g* for 5 minutes.
7. Soluble proteins will be in the Receiver Plate. Remove the Filter Plate and set aside. (Do not place it on an absorbent surface if you want to recover the insoluble proteins.) Analyze 15 µl of the soluble proteins by SDS-PAGE.

After you have isolated the soluble proteins, insoluble proteins will remain in the Filter Plate. Use the following steps to recover these proteins:

8. Place the Filter Plate containing your insoluble protein on top of a new Receiver Plate.
9. Add 200 µl of Denaturing Buffer to each well of the Filter Plate and incubate for 10 minutes.
10. Centrifuge the stacked plates at 2000 × *g* for 5 minutes.
11. Insoluble proteins will be in the Receiver Plate. Discard the Filter Plate. Analyze 15 µl of the insoluble proteins by SDS-PAGE.

Insect Cells

Introduction

This section provides protocols for isolating soluble and insoluble proteins from insect cells.

Isolating Proteins from Insect Cells

Use the following protocol to recover soluble proteins from insect cells. (Do not add Lysozyme to the Lysis Buffer for insect cells.)

Note: Do not place the Filter Plate on any absorbent surface during the protocol to prevent the loss of sample.

1. Place the Filter Plate on top of a Receiver Plate. Transfer 150 µl of cell culture to each well of the Filter Plate.
2. Add 50 µl of Lysis Buffer to each well of the Filter Plate. All of the Lysis Buffer may not absorb into the filter. Do not mix by pipetting.
3. Incubate the stacked plates at room temperature for 10–15 minutes.
4. Centrifuge the stacked plates at 1000–2500 × *g* for 5 minutes at room temperature. You may centrifuge the plates at +4° C if your protein is sensitive to high temperature.
5. Soluble proteins will be in the Receiver Plate. Remove the Filter Plate and set aside. (Do not place it on an absorbent surface if you want to recover the insoluble proteins.) Analyze 15 µl of the soluble proteins by SDS-PAGE.

After you have isolated the soluble proteins, insoluble proteins will remain in the Filter Plate. Use the following steps to recover these proteins:

6. Place the Filter Plate containing your insoluble protein on top of a new Receiver Plate.
7. Add 200 µl of Denaturing Buffer to each well of the Filter Plate. All of the Denaturing Buffer may not absorb into the filter.
8. Incubate the stacked plates at room temperature for 10 minutes.
9. Centrifuge the stacked plates at 1000–2500 × *g* for 5 minutes at room temperature. You may centrifuge the plates at +4°C if your protein is sensitive to high temperature.
10. Insoluble proteins will be in the Receiver Plate. Discard the Filter Plate. Analyze 15 µl of the insoluble proteins by SDS-PAGE.

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Phosphate Buffer

For Step 3 of the following protocol, prepare a simple Phosphate Buffer of 50 mM phosphate and 100 mM NaCl at pH 8.0. Prepare 150 µl of buffer per 1 ml of cell culture.

Isolating Proteins with Low Expression Levels from Insect Cells

The following protocol can be used to isolate soluble and insoluble proteins with low expression levels from insect cells.

The amounts in the protocol are per well. Scale up as needed.

Note: Do not place the Filter Plate on any absorbent surface during the protocol to prevent the loss of sample.

1. Centrifuge 1 ml of cell culture at 3000 × *g* or higher for 10 minutes to pellet the cells.
2. Remove all of the media by aspiration or tapping the container upside down.
3. Add 150 µl of the Phosphate Buffer described above to the cell pellet.
4. Resuspend the cells in the buffer by vortexing briefly or pipetting up and down.
5. Place the Filter Plate on top of a Receiver Plate. Add 150 µl of cell solution to each well of the Filter Plate.
6. Add 50 µl of Lysis Buffer to each well of the Filter Plate and incubate for 10–15 minutes at room temperature. All of the Lysis Buffer may not be absorbed into the filter. Do not mix by pipetting.
7. Centrifuge the stacked plates at 2000 × *g* for 5 minutes.
8. Remove the Filter Plate. (Do not place it on an absorbent surface if you want to recover the insoluble proteins.) Analyze 15 µl of the soluble protein in the Receiver Plate by SDS-PAGE.

After you have isolated your soluble proteins, insoluble proteins will remain in the Filter Plate. Use the following steps to recover these proteins:

9. Place the Filter Plate containing your insoluble protein on top of a new Receiver Plate.
10. Add 200 µl of Denaturing Buffer to each well of the Filter Plate and incubate for 10 minutes.
11. Centrifuge the stacked plates at 2000 × *g* for 5 minutes.
12. Discard the filter plate. Analyze 15 µl of the insoluble protein in the receiver plate by SDS-PAGE.

Troubleshooting

Problem	Cause	Solution
Clogged filter	Cell density is too high	Reduce the cell density. Avoid loading bacterial cultures with an $OD_{600} > 15$.
Low protein yield	Bacterial cells—poor induction of expression	If you are using a 96-well growth block, induction may be difficult and slow; we recommend growing bacterial cell cultures in 24- or 48-well growth blocks for optimal expression.
	Low protein expression	For proteins with low expression levels, use the **Isolating Proteins with Low Expression Levels from Bacterial Cells** protocol on page 5 or the **Isolating Proteins with Low Expression Levels from Insect Cells** protocol on page 7.
	Low protein expression or protein may be toxic to cells	Optimize growth conditions or try a different expression system to increase protein expression.

License Information

Limited Use License Number 94: Protein Screen 96 System

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Technical Service, Continued

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