Invent ® High Throughput Spin Column-Based Total Protein Extraction Kit for Cells (96 Wells)

Cat. No. IN-WB96001

Description:

Invent ® High Throughput Spin Column-Based Total Protein Extraction Kit for Cells (96 Wells) is a high-throughput super-fast protein extraction tool. More and more evidences have shown that the most commonly used RIPA buffer can cause unpredictable protein loss, resulting in questionable data interpretation. This problem is fully resolved using the spin column-based technologies. Coupled with stronger lysis buffers, proteins can be extracted more efficiently. The High Throughput Spin Column-Based Total Protein Extraction Kit can extract protein from cultured cells in 96-well plate, or non-96 well cultured cell simultaneously. It is simple, fast and high yield.

Application:

Invent ® High Throughput Spin Column-Based Total Protein Extraction Kit for Cells (96 Wells) is designed to rapidly extract total proteins for applications such as SDS-PAGE, WB, ELISA.

Kit components (96 tests/plate):

1. Denaturing Cell Lysis Buffer 25 ml×2
2. 96-Well Filter Cartridges Plate 1
3. 96-Well Collection Plate 1
4. 96-Well Rubber Pad 1
5. Adhesive Plate Seal 1

NOTE: **Cell lysis buffers listed above do not contain any reducing agents and primary amine

Shipping: This kit is shipped at ambient temperature

Storage: Store the kit at room temperature

Important Product Information:

Invent ® High Throughput Spin Column-Based Total Protein Extraction Kit for Cells (96 Wells) is designed to rapidly extract total proteins. The use of protease inhibitors is optional. However, if downstream application takes significant amounts of time or the extract protein will be stored for a more extended period, the addition of protease inhibitors to cell lysis buffer is recommended. For determination of protein concentration, BCA kit is recommended. For protein phosphorylation studies, phosphatase inhibitors (e.g., PhosStop from Roche) should be added to the lysis buffer before use.

*****If precipitate is found in Denaturing Buffer at lower temperature, incubate at >37^{0}C until the precipitate is completely dissolved.***

Additional Materials Required:

1 X PBS

Plate Centrifuge

Protocols:

Total Protein Extraction for 96-well Cultured Cells

1. Place the 96-well filter cartridges plate in the 96-well collection plate. Pre-chill the set of plates on ice.
2. For adherent cells(96-well), Grow cells to 90-100% confluence and discard the medium. Wash the cells with cold PBS once. Aspirate the supernatant completely. A multi-channel pipetting device could be used.
3. Add 25-40ul denaturing cell lysis buffer to each well of the cell sample in the 96-well plate, Pipette repeatedly to lyse the cells with a multi-channel. Transfer the cell lysate to the pre-chill 96-well filter cartridges plate with 96-well collection plate. Capped the 96-well filter cartridges plate with the adhesive plate seal. Centrifuge at 4000rpm for 5 minutes at 4℃ using the plate centrifuge.
4. Discard the 96-well filter cartridges plate. The sample in the 96-well collection plate is now ready for downstream applications. The sample can be covered with 96-well rubber pad and frozen at -80℃ for further analysis. Thaw the sample on ice and centrifuge at 4000rpm for 5min at 4℃ before use.

Total protein extraction from non-96 well cultured cells

1. Place the 96-well filter cartridges plate in the 96-well collection plate. Pre-chill the set of plates on ice.
2. **For adherent cells:** Grow cells to 90-100% confluence and wash the cells with cold PBS. Aspirate the buffer completely. Collect cells into a 1.5ml tube using Trypsin or cell scraper. Centrifuge at 700Xg for 5min at 4℃. Aspirate the supernatant completely.

   For non-adherent cells: Harvest cells by low-speed centrifugation. Wash the cells with cold PBS once in a 1.5 ml microcentrifuge tube and pellet the cells by centrifugation at 500 X g for 2-3 min. Aspirate the supernatant and leave a small amount of PBS (about the volume of packed cells) in the tube. Vortex briefly to resuspend the cells.
3. Add appropriate amounts of cell lysis buffer to the cell suspension (Table 1) and vortex briefly to lyse the cells.

   Note: the presence of small amount of un-lysed cells would not affect the quality of the samples.

Table 1. Lysis Buffer Volume for Different Packed Cell Volumes

Packed cell volume (μl)	lysis buffer (μl)
3	20
5	50
10	100
20	200
40	500

Note: this is a general reference, the actual amount of lysis buffer can be more or less

4. Mark the samples and transfer the cell lysate to the pre-chill 96-well filter cartridges plate with 96-well collection plate in sequence. Capped the 96-well filter cartridges plate with the adhesive plate seal. Centrifuge at 4000rpm for 5 minutes at 4℃ using the plate centrifuge.
5. Discard the 96-well filter cartridges plate. The sample in the 96-well collection plate is now ready for downstream applications. The sample can be covered with 96-well rubber pad and frozen at -80℃ for further analysis. Thaw the sample on ice and centrifuge at 4000rpm for 5min at 4℃ before use.

For Research Use Only. Not for use in diagnostic procedures.