Minute™ Golgi Apparatus Enrichment Kit

Cat. No. GO-037

Description

The Golgi apparatus, also known as the Golgi complex or Golgi body, consists of a series of flattened stacked pouches called cisternae. This organelle plays a crucial role in eukaryotic cells by facilitating the transportation, modification, and packaging of proteins and lipids into vesicles for delivery to specific locations. The quantity and distribution of Golgi vary significantly across different cell and tissue types. Obtaining a highly enriched Golgi fraction is a crucial initial step in the study of its function and interactions with other organelles.

Traditional methods for isolating the Golgi apparatus rely on density gradient ultracentrifugation, which demands a substantial amount of starting material and can be laborious and time-consuming. In contrast, the Minute™ kit distinguishes itself from other Golgi isolation kits by employing patented spin-column-based technology. This approach is both straightforward and rapid, requiring only a small amount of starting material. With this kit, native Golgi can be preferentially enriched through precipitation, eliminating the need for a Dounce homogenizer and ultracentrifugation. It enables the isolation of two sub-Golgi fractions: the Golgi apparatus and secretory vesicles of the Golgi.

Kit Components (20 Preps) :

1.	Buffer A	20 ml
2.	Buffer B	8 ml
3.	Buffer C	2 ml
4.	Buffer D	2 ml
5.	Plastic rods	2
6.	Filter Cartridge	20
7.	Collection Tube	20

Additional Materials Required

1 X PBS, Vortexer, Table-Top Micro centrifuge with maximum speed of 16,000X g. **The centrifuge should be able to reach maximum speed within 10 seconds.**
Shipping and Storage: Ship at ambient temperature and store at 4ºC.

Important Information:

1. Carefully review the entire procedure. Prior to use, chill the filter cartridge along with the collection tube on ice. This kit is primarily designed for enriching Golgi from liver tissue. Its suitability for other tissues may vary.
2. Ensure that all centrifugation steps are carried out at 4°C, either in a cold room or using a refrigerated microfuge.
3. If your research involves protein phosphorylation, it's essential to add phosphatase inhibitors (e.g., PhosStop from Roche) to an aliquot of buffer A before usage. For concerns related to protein degradation, include protease inhibitor cocktails in buffer A before use.

4. We recommend using the BCA Protein Assay Kit for determining protein concentration
5. Please note that the yield and purity of the isolated Golgi apparatus may vary depending on the specific cell/tissue types and the amount of starting material used. The protocol may require optimization to achieve the best results (refer to the technical notes below).

Protocol

Note: Warm buffer B to room temperature and mix well prior to use.

1. Begin by placing the filter cartridges in a collection tube and incubating them on ice.
2. **For cultured cells:** collect 25-35 X 10^6 cells through low-speed centrifugation (500-600 X g for 5 min). Wash the cells once with cold PBS and ensure complete removal of the supernatant. Resuspend the pellet in 550 µl of buffer A. Vigorously vortex the tube for 20-30 seconds, then promptly transfer the cell suspension to a filter cartridge. Proceed to step 3.
   For tissue samples: place 30-40 mg of tissue (whether fresh or frozen) in a filter cartridge with a collection tube. Add 200 µl of buffer A to the filter and grind the tissue with a plastic rod for 2-3 minutes by repeatedly pushing the tissue against the filter's surface with a twisting force. After grinding, add 350 µl of buffer A to the same filter cartridge, mix by pipetting up and down a few times, and move to step 3. The plastic rod is reusable and can be cleaned with 70% alcohol or water.
3. Cap the filter cartridge, invert the tube several times, and centrifuge at 16,000 X g for 30 seconds.
4. Centrifuge the tube at 4°C for 5 minutes at 5,000 X g without removing the filter. After centrifugation, remove the filter and transfer all the supernatant to a fresh 1.5 ml tube (try to minimize the inclusion of lipids as much as possible) and centrifuge at 4°C for 30 minutes at 16,000 X g. Following centrifugation, carefully transfer 400 µl of the supernatant to a fresh 1.5 ml tube. The pellet primarily contains mitochondria, endoplasmic reticulum (ER), lysosomes, and plasma membranes.
5. Add 400 µl of buffer B to the tube containing 400 µl of supernatant. Mix well by briefly vortexing (the buffer B to supernatant ratio is 1:1). Incubate the tube on ice for 10 to 15 minutes. Centrifuge at 8,000 X g for 5 minutes.
6. Transfer the supernatant to a fresh tube, which contains secretory vesicles of the trans-Golgi. To concentrate this fraction, refer to step 10 below. Resuspend the pellet in 200 µl of cold buffer A by pipetting up and down 40-50 times. Centrifuge at 8,000 X g for 5 minutes.
7. Transfer the supernatant to a fresh 1.5 ml tube. Add 100 µl of cold buffer C to the supernatant and mix by vigorously vortexing for 20 seconds. Incubate the tube on ice for 20 minutes.
8. Centrifuge the tube at 8,000 X g for 10 minutes. Remove and discard the supernatant. Spin the tube at 8,000 X g for a few seconds to bring down any residual reagent on the tube's walls and remove it completely. The pellet contains the enriched Golgi.
9. Resuspend the pellet in 50 to 200 µl of a detergent-containing buffer. If the preparation is not used immediately, add protease inhibitor cocktails to the preparation and store it at -80°C. The typical Golgi yield ranges from 50 to 200 µg per sample. The water-insoluble Golgi fraction can be dissolved in a buffer of your choice, but the following reagents (see the table below) are recommended based on your downstream applications.
10. To concentrate trans-Golgi secretory vesicles, add 100 µl of buffer D to the supernatant from step 6, mix well by vortexing for 10 seconds, incubate on ice for 20 minutes, and centrifuge at 16,000 X g for 5 minutes. Remove and discard the supernatant entirely. Centrifuge briefly to bring down any residual reagent and remove it completely. Resuspend the pellet in 50-100 µl of the reagent listed in the table below or in a buffer of your choice.

Tech Notes:

1. This kit segregates the Golgi apparatus into two distinct sub-fractions: one consisting of mainly the cis-Golgi and the other containing secretory vesicles with minimal cross-contamination from the cis-Golgi.
2. The enriched Golgi fraction obtained primarily contains cis-Golgi markers like GM-130, along with some trans-Golgi markers such as Clint1 and Golgin97.
3. The secretory vesicle fraction from step 10 contains trans-Golgi markers like TGN46, TGN38, and Clint1.
4. If the Golgi pellet is not efficiently solubilized using WA-009 (as listed in the table below), consider adding SDS to WA-009 to reach a final concentration of 0.4% and increasing the volume of the protein solubilization reagent. It's important to note that certain components in buffer C might interfere with mass spectrometry analysis and should be removed after trypsin digestion.
5. To evaluate the yield and purity of the isolated Golgi, we recommend comparing it to the total cell/tissue lysate in Western blotting (WB) using an antibody specific to Golgi, such as GM130. Ensuring equal protein loading in SDS-PAGE is crucial. Staining the post-transfer blot with Ponceau Red can provide valuable insights into potential variations in protein loading.
6. The extent of Golgi enrichment is contingent on the type of sample. Intracellular membranous structures are interconnected, and it's not uncommon to detect certain cytosolic marker proteins like actin and tubulin associated with organelles in the isolated Golgi apparatus.
7. Keep in mind that budding secretory vesicles may only be partially depleted from the cis-Golgi fraction, and the efficiency of precipitating budding secretory vesicles in step 10 can vary significantly depending on the cell or tissue type.
8. The Golgi yield primarily depends on two factors: A. the amount of starting material and B. the efficiency of cell membrane rupture. You can assess the rupture efficiency by staining cells with trypan blue before and after passing them through the filter. Cell viability should be over 90% before and less than 30% after passing through the filter. If cell rupture efficiency is low, the solution is to freeze and thaw cells, resuspend cells in buffer A and freeze and thaw at -80°C twice, and then follow the standard protocol.
9. If the isolated Golgi fraction does not exhibit the expected enrichment, you should examine the following fractions in WB: Total cell lysate, supernatant, and pellet in step 4 (after the 16,000 X g spin), the pellet from step 5, the supernatant and pellet of step 8. Analyzing these results will provide insights for protocol optimization.

The following protein solubilization reagents are recommended.

Product Name	Cat. No.	Applications
Minute™ Denaturing Protein Solubilization Reagent	WA-009	SDS-PAGE electrophoresis and Western blotting, trypsin digestion, purification of proteins with biotin labeling or histidine labeling, etc.
Minute™ Non-Denatured Protein Solubilization Reagent	WA-010	ELISA, immunoprecipitation/Co-IP, enzymatic activity determination and other applications.
Minute™ Protein Solubilization Reagent for MS	WA-011	Trypsin digestion and subsequent mass spectrometry analysis.