Minute™ Golgi Apparatus Enrichment Kit

Cat. No. GO-037

Description

Golgi apparatus, also called Golgi complex or Golgi body, is made up of a series of flattened stacked pouches (cisternae). Golgi apparatus is vital organelle in eukaryotic cells and is responsible for transporting, modifying, and packaging protein and lipids into vesicles for delivery to targeted locations. The quantity and distribution of Golgi in different cell and tissue types vary significantly. The ability to obtain highly enriched Golgi fraction is an important first step for studying its function and interaction with other organelles. Traditional methods for isolating Golgi apparatus is based on density gradient ultracentrifugation. The protocol requires large amount of starting material and the methods are tedious and time consuming Unlike any other Golgi isolation kit, this kit employees a patented spin-column based technology that is simple, rapid and requires only small amount of starting material. This kit can preferentially enrich native Golgi by precipitation without using a Dounce homogenizer and ultracentrifugation. The whole protocol can be completed in < 2h.

Kit Components (20 prep)

Buffer A	20 ml
Buffer B	8 ml
Buffer C	2 ml
Plastic rods	2
Filter Cartridge	20
Collection Tube	20

Additional Materials Required

1 X PBS, Vortexer, Table-Top Micro centrifuge with maximum speed of 16,000 rpm.
The centrifuge should be able to reach maximum speed within 10 seconds.

Shipping and Storage: Ship and store at room temperature

Important Information:

1. Read the entire procedures carefully. Chill protein extraction filter cartridge with collection tube on ice prior to use. This kit is particularly designed for enrich Golgi from liver tissue. Other tissue may or may not be suitable.
2. **All centrifugation steps should be performed at 4°C in a cold room or in a refrigerated microfuge.**

Invent Biotechnologies, Inc., 1864 Berkshire Ln, N, Plymouth MN 55441
Tel: (866)-817-7611, Fax: 952-400-6889, www.inventbiotech.com

3. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to aliquot of buffer A prior to use. If protein degradation is a concern, add protease inhibitor cocktails to buffer A prior to use.
4. It is recommended to use BCA Protein Assay Kit for determination of protein concentration (Pierce, Cat #:23227).

Protocol

Important Note: The amounts of Golgi apparatus in a given cell type or tissue vary significantly. For demonstration purpose, liver tissue was used as starting material. Other cell/tissue may or may not be suitable. The performance of the protocol is cell/tissue type-dependent.

1. Place the filter cartridges in a collection tube and incubate on ice.

A. **For cultured cells**, collect 25-35 X 10^6 cells by low speed centrifugation (500-600 X g for 5 min). Wash cells once with cold PBS. Remove supernatant completely. Resuspend the pellet in 550 µl buffer A. **Vortex the tube vigorously for 20-30 seconds**. Immediately transfer the cell suspension to a filter cartridge. Go to step 2.

B. **For tissue samples** place 30-40 mg tissue (fresh or frozen) in a filter cartridge with collection tube. Add 200 µl buffer A to the filter and grind the tissue with a plastic rod for 2-3 min by pushing the tissue against the surface of the filter repeatedly with twisting force After grinding, add 350 µl buffer A to the same filter cartridge, mix by pipette up and down a few times and go to step 2. **The plastic rod is reusable. Clean it with 70% alcohol or water.**

2. Cap the filter cartridge, invert the tube a few times and centrifuge at 16,000 X g for 30 seconds.

3. Centrifuge the tube at 4°C for 5 min at 5,000 X G without removing the filter. After centrifugation, remove the filter and transfer all supernatant to a fresh 1.5 ml tube (try to avoid lipids as much as possible) and centrifuged at 4°C for 30 min at 16,000 X g. After centrifugation, carefully transfer 400 µl supernatant to a fresh 1.5 ml tube. The pellet contains mainly mitochondria, ER, lysosomes and plasma membranes.

4. Add 400 µl buffer B to the tube containing 400 µl supernatant. Mix well by vortexing briefly (buffer B to supernatant ratio is 1:1). Incubate the tube on ice for 10 to 15 min. Centrifuge at 8,000 X G for 5 min.

5. Remove supernatant completely and resuspend the pellet in 200 ul cold buffer A by pipetting up and down for 40-50 times. Centrifuge the tube at 8,000 X G X g for 5 min.

Invent Biotechnologies, Inc., 1864 Berkshire Ln, N, Plymouth MN 55441
Tel: (866)-817-7611, Fax: 952-400-6889, www.inventbiotech.com

6. Transfer the supernatant to a fresh 1.5 ml tube. Add 100 µl cold buffer C to the supernatant and mix by vortexing vigorously for 20 seconds. Incubate the tube on ice for 20 min.

7. Centrifuge the tube at 8,000 X g for 10 min. Remove and discard the supernatant. Spin the tube at 8,000 X g for a few second to bring down the residual reagent on the wall of the tube. Remove it completely.

8. Resuspend the pellet in 50 to 200 µl PBS (this is isolated Golgi fraction, if the prep is not used right away, add protease inhibitor cocktails to the prep and store at -80ºC). The Golgi yield is typically 50-200 µg/sample. The water insoluble Golgi fraction can be dissolved in any buffer of your choice but following reagents are recommended depending upon downstream applications.

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.

Invent Biotechnologies, Inc., 1864 Berkshire Ln, N, Plymouth MN 55441
Tel: (866)-817-7611, Fax: 952-400-6889, www.inventbiotech.com