Product Information

Golgi Isolation Kit

Sufficient for 50 g (tissue)

GL0010

Product Description

Storage Temperature: 2-8 °C

The Golgi apparatus consists of a series of flattened membrane vesicles. The stacks of flattened cisternae have three defined regions called the cis-Golgi, the medial Golgi, and the trans-Golgi. Transport vesicles that contain newly synthesized proteins move from the endoplasmic reticulum (ER) by fusing with the cis-Golgi cisternae, delivering proteins for further Golgi modifications. These proteins then progress from the cis-Golgi, through the medial Golgi, to the trans-Golgi. On the way, the proteins are subjected to different modifications depending on their structure and destination. Modified proteins are transported out of the Golgi apparatus in coated vesicles that bud off the trans-Golgi cisternae.[1]

The Golgi Isolation Kit provides a method for isolating Golgi membranes from mammalian soft tissues by a discontinuous density gradient.[2-6] The degree of Golgi enrichment can be determined by assaying the activity of UDP-galactosyl transferase[7] or by immunodetection of Golgi specific marker proteins like β-COP or GM130 with appropriate antibodies (Cat. Nos. G6160 and G7295, respectively). Separation from other organelles can be measured using the appropriate marker detection kits (see 'Additional Kits for measuring enzymatic activities').

The kit was optimized using rat liver and tested on rat kidney, spleen, and heart. Several dissertations[8-11] have cited use of product GL0010 in their protocols.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Components

The reagents are sufficient for Golgi isolation from 50 grams of tissue.

- Isolation Buffer, 5× (Component I4534): 120 mL
- 2.3 M Sucrose Solution (Component S6571): 120 mL
- Protease Inhibitor Cocktail, for use with mammalian cell and tissue extracts (Component P8340): 5 mL

Reagents and Equipment Required

(Not provided)

- Ultrapure water (17 MΩ·cm or equivalent)
- PBS (Phosphate Buffered Saline, such as Cat. No. D8537)
- Ultracentrifuge with fixed angle or swinging bucket head capable of 150,000 × g and appropriate tubes
- Tissue homogenizer: Overhead electric motor together with Potter-Elvehjem PTFE pestle in glass tube homogenizer
- Scalpel, forceps, and glass plate
- Microcentrifuge tubes
- Pasteur pipettes
- Additional Kits for measuring enzymatic activities:
  - β-N-Acetylglucosaminidase Assay Kit (Cat. No. CS0780): β-N-Acetylglucosaminidase is a lysosomal marker
  - Cytochrome c Oxidase Assay Kit (Cat. No. CYTOCOX1): Cytochrome c oxidase is a mitochondrial marker
  - Cytochrome c Reductase Assay Kit (Cat. No. CY0100): Cytochrome c reductase is an endoplasmic reticulum (ER) marker
  - Catalase Assay Kit (Cat. No. CAT100): Catalase is a peroxisomal marker

Storage/Stability

- The kit is shipped on cooler packs (wet ice).
- After receiving the kit, the Protease Inhibitor Cocktail (Component P8340) should be stored in working aliquots at –20 °C.
- The 2.3 M Sucrose Solution (Component S6571) and the Isolation Buffer, 5× (Component I4534) can be stored at 2-8 °C. However, to avoid contamination of these solutions, they can be stored at –20 °C, especially after opening the bottles.
- The components are stable for 24 months when stored unopened at the appropriate temperatures.

Preparation Instructions

It is recommended to use ultrapure (17 MΩ·cm or equivalent) water when preparing the reagents. Prior to use, thaw the kit components and mix until homogeneous.

- 1× Isolation Buffer: Dilute an aliquot of the Isolation Buffer, 5× (Component I4534) 5-fold with water. 60 mL of 1× Isolation Buffer are required for 5 grams of tissue.
- Sucrose Solutions: Prepare sucrose solutions at different concentrations for a sucrose gradient by diluting the 2.3 M Sucrose Solution (Component D6571) with 1× Isolation Buffer. The following volumes are suitable for preparing a gradient for Golgi isolation from ~5 grams of tissue:
  - 45 mL of 0.25 M Sucrose Solution
  - 20 mL of 1.1 M Sucrose Solution
  - 10 mL of 1.84 M Sucrose Solution

Supplement all the Sucrose Solutions with Protease Inhibitor Cocktail to a 1% (v/v) final concentration.

Procedure

Isolation of Golgi membranes from rat liver (~5 grams of tissue)

Notes:

- The procedure can be scaled up or down.
- The procedure can be used for other tissues. However, the homogenization conditions (strokes, speed, amount of buffer, etc.) must then be optimized.
- The use of a homogenization device other than PTFE in glass (detailed in the Reagents and Equipment Required but Not Provided section) is possible but requires optimization.
- Perform the whole procedure at 2-8 °C. All the solutions and equipment should be pre-cooled before use.

1. Use a fresh tissue sample from an animal that was starved overnight and sacrificed the following morning.
   - It is possible to use a flash frozen sample. However, the Golgi fraction isolated may be more contaminated with other organelles, and some enzyme activities may be affected compared to a sample prepared using fresh tissue.
2. Wash the tissue with ice-cold PBS or any other isotonic buffer.
3. Then wash the tissue briefly with 10 mL of 0.25 M Sucrose Solution.
4. Weigh the tissue.
5. Mince the tissue with scissors. If excessive blood is observed, wash the tissue once again with 5-10 mL of 0.25 M Sucrose Solution.
6. Suspend the minced tissue with 1 mL of the 0.25 M Sucrose Solution per 1 g of tissue.
7. Transfer the tissue suspension into the appropriate homogenization tube.
   7.1. Homogenize with 6 slow motions of the PTFE pestle at 300 rpm.
8. Transfer the homogenate into a centrifuge tube.
   8.1. Centrifuge at 3,000 × g for 15 minutes at 2-8 °C.
   8.2. Transfer the supernatant to a fresh tube.
9. Calculate the sucrose concentration in the supernatant according to Equation 1:

Equation 1:

$$[\text{Sucrose}] = \frac{(0.25 \times \text{Buffer Volume})}{(\text{Tissue Weight} + \text{Buffer Volume})}$$

Where:

- [Sucrose] = molar sucrose concentration in the supernatant
- 0.25 = molar sucrose concentration in the homogenization buffer
- Buffer Volume = volume (mL) of the buffer used to resuspend tissue (Step 6)
- Tissue Weight = weight of tissue in grams (Step 4)

10. Calculate the volume of the 2.3 M Sucrose Solution (Component D6571) to be added to the supernatant to obtain a final sucrose concentration of 1.25 M according to Equation 2:

Equation 2:

$$\text{Sucrose Volume} = \frac{\{\text{Sup Volume} \times (1.25 - [\text{Sucrose}])\}}{1.05}$$

Where:

- Sucrose Volume = volume (mL) of 2.3 M Sucrose Solution to be added to the supernatant, in order to obtain a final concentration of 1.25 M sucrose.
- Sup Volume = volume (mL) of the supernatant
- [Sucrose] = molar sucrose concentration in the supernatant (from Equation 1).
- 1.05 = the difference in the molarity between the sucrose stock solution (2.3 M) and the sucrose concentration of the sample (1.25 M).

11. Adjust the sucrose concentration in the sample (supernatant) to 1.25 M by adding the volume of 2.3 M Sucrose Solution calculated in Equation 2.
    11.1. Mix by inverting the tube.
    11.2. Vortex briefly.
12. Build a discontinuous gradient in an ultracentrifuge tube. The order of sucrose gradient fractions in the tube (from bottom to top) should be:
    - 1.84 M Sucrose Solution
    - Sample (sucrose concentration adjusted to 1.25 M)
    - 1.1 M Sucrose Solution
    - 0.25 M Sucrose Solution

Notes:

- The total volume of the separation gradient can be in the range of 4-50 mL. The gradient volume will dictate the amount of sample to be loaded on the gradient. Examples are given in Table 1.
- The ratio between the volumes of the different sucrose fractions in the gradient depends on the type of rotor used. Table 1 summarizes the suggested ratios for gradient preparation in a swinging or a fixed angle rotor. The sample volume is assigned a relative volume of 1.

Table 1. Golgi Density Gradient Preparation

Gradient Fraction	Relative Volumes		Suggested Volumes (mL)	
	Swinging angle rotor	Fixed angle rotor	Swinging (SW50.1) 4-5 mL tube	Fixed (Ti45) 60 mL tube
1.84 M Sucrose (bottom)	0.3-0.5	1	0.5	10
Sample (1.25 M Sucrose)	1	1	1.5	10
1.1 M Sucrose	0.7-1.5	1.7-2	1	20
0.25 M Sucrose (top)	0.5-1	0.5-1	1	10

13. Centrifuge the tubes at 120,000 × g for 3 hours at 2-8 °C.
14. Withdraw the Golgi enriched fraction from the 1.1 M/0.25 M sucrose interphase.

The storage conditions for the enriched Golgi fraction depend on the required application. For example, the fraction can be snap-frozen for further detection of the enzymatic activities listed in the Reagents and Equipment Required (Not Provided) Section.

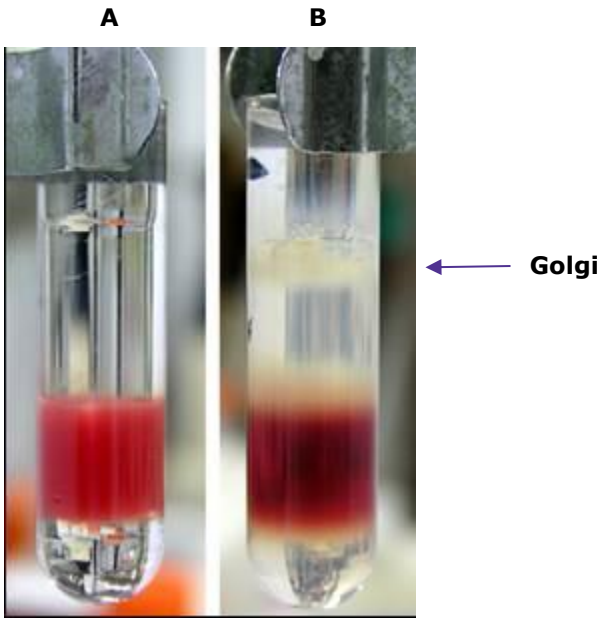
Results

Three bands will be visualized on the gradient:

- First band: at the 1.84 M/1.25 M interphase
- Second band: at the 1.25 M/1.1 M interphase
- Third band: at the 1.1 M/0.25 M interphase

In addition, there may be a lipid layer floating on the very top of the gradient. Figure 1 illustrates an example.

Figure 1. Golgi Density Gradient



Rat liver homogenate was prepared according to the procedure for this kit. The 3,000 × g supernatant containing sucrose (1.25 M) was used in building a step density gradient.

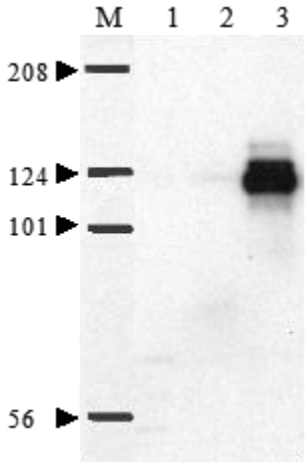
A: The density gradient before centrifugation

B: The density gradient after centrifugation

The arrow indicates the position of the Golgi-enriched fraction. The Golgi-enriched fraction is located at the 1.1 M/0.25 M sucrose interphase.

The enrichment of the Golgi fraction is demonstrated by detection of the Golgi marker, GM130 (see Figure 2).

Figure 2. Detection of the Golgi marker, GM130, during the different steps of Golgi enrichment



The Golgi-enriched fraction was isolated from rat kidney using the Golgi Isolation Kit following the described procedure:

1. 15 µg of total protein from each step were separated on a 7.5% SDS-PAGE gel:
   - M: marker
   - Lane 1: homogenate
   - Lane 2: 3000 × g supernatant
   - Lane 3: Golgi gradient-enriched fraction
2. An immunoblot was performed using anti-GM130 antibody (Cat. No. G7295).

References

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GL0010pis Rev 08/22 GL,EB,GCY,MAM