

Golgi Isolation Kit

Cat. No. GGI-322K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Golgi Isolation Kit may be used for the isolation of Golgi membranes from mammalian soft tissues by discontinuous density gradient.
Usage	1 Kit is sufficient for 50 g (tissue)
Storage	The kit is shipped on wet ice. After receiving the kit, the Protease Inhibitor Cocktail should be stored in working aliquots at -20 centigrade. The 2.3 M Sucrose Solution and the Isolation Buffer, 5× can be stored at 2-8 centigrade. However, in order to avoid contamination of these solutions they can be stored at -20 centigrade, especially after opening the bottles. The components are stable for 24 months when stored unopened at the appropriate temperatures.
Kit Components	Isolation Buffer, 5×120 ml 2.3 M Sucrose Solution 120 ml Protease Inhibitor Cocktail for use with mammalian cell and tissue extracts 5 ml
Materials Required but Not Supplied	· Ultrapure water (17 MW×cm or equivalent) · PBS · 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

 Tel: 1-631-559-9269 1-516-512-3133

 Email: info@creative-biomart.com  Fax: 1-631-938-8127

 45-1 Ramsey Road, Shirley, NY 11967, USA

- Microcentrifuge tubes
- Pasteur pipettes
- Additional Kits for measuring enzymatic activities:
 - b-N-Acetylglucosaminidase Assay Kit- b-N-Acetylglucosaminidase is a lysosomal marker.
 - Cytochrome c Oxidase Assay Kit - Cytochrome c oxidase is a mitochondrial marker.
 - Cytochrome c Reductase Assay Kit - Cytochrome c reductase is an endoplasmic reticulum (ER) marker.
 - Catalase Assay Kit - Catalase is a peroxisomal marker.

Preparation

It is recommended to use ultrapure (17 MW×cm or equivalent) water when preparing the reagents.

Prior to use, thaw the kit components and mix until homogeneous.

1×solation Buffer - Dilute an aliquot of the Isolation Buffer, 5×, 5-fold with water. Sixty 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 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 [1% (v/v) final concentration].

Separation Protocol

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

Notes:

- The procedure can be scaled up/down.
- The procedure can be used for other tissues; however, the homogenization conditions (strokes, speed, amount of buffer, etc.) must 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 centigrade. 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. Wash the tissue briefly with 10 ml of 0.25 M Sucrose Solution.

3. Weigh the tissue.

4. 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.

5. Suspend the minced tissue with 1 ml of the 0.25 M Sucrose Solution per 1 g of tissue.

6. Transfer the tissue suspension into the appropriate homogenization tube and homogenize with 6 slow motions of the PTFE pestle at 300 rpm.

7. Transfer the homogenate into a centrifuge tube and centrifuge at 3,000× g for 15 minutes at 2–8 centigrade. Transfer the supernatant to a fresh tube.

8. Calculate the sucrose concentration in the supernatant according to Equation 1:

Equation 1. $[\text{Sucrose}] = (0.25 \times \text{Buffer Volume}) / (\text{Tissue Weight} + \text{Buffer Volume})$

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 5)

Tissue Weight – weight of tissue in grams (step 3)

9. Calculate the volume of the 2.3 M Sucrose Solution to be added to the supernatant to obtain a final sucrose concentration of 1.25 M according to Equation 2:

Equation 2. $\text{Sucrose Volume} = (\text{Sup Volume} \times (1.25 - [\text{Sucrose}])) / 1.05$

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).

10. 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. Mix by inverting the tube followed by a brief vortex.

11. 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

12. Centrifuge the tubes at 120,000 ×g for 3 hours at 2–8 centigrade.

13. 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 but Not Provided Section.