# Golgi Apparatus Staining Kit

# User Manual

## Catalog # CAK2011

(Version 1.1A)

For selectively staining the Golgi apparatus in live or aldehyde fixed cells

**For research use only. Not for diagnostic or therapeutic procedures.**

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## I. INTRODUCTION

The Golgi apparatus is a complex of vesicles and folded membranes within the cytoplasm of most eukaryotic cells, involved in secretion and intracellular transport. It modifies proteins and lipids that have been built in the endoplasmic reticulum (ER) and prepares them for export outside of the cell. It also plays a significant role in the transport of lipids throughout the cell and the formation of lysosomes.

## II. PRINCIPLES

Golgi Apparatus Staining Kit provides a simple and rapid way to stain Golgi in live cells, or aldehyde-fixed cells selectively. C6 NBD Ceramide is administered to cells as a complex with bovine serum albumin (C6-NBD-Ceramide-BSA). Golgi apparatus is stained through the formation of the respective fluorescent metabolites. Golgi Apparatus Staining Kit provides an optimized assay method for examining the morphology of the Golgi apparatus with a fluorescence microscope.

## III. KIT COMPONENTS

| Component | 100 Assays | 500 Assays | Storage |
|---|---|---|---|
| C6 NBD Ceramide | Powder x 1 | Powder x 5 | -20 °C |
| Diluent | 1 ml x 1 | 1 ml x 2 | RT |
| BSA Solution | 4 ml x 1 | 20 ml x 1 | -20 °C |
| Binding Buffer | 0.2 ml x 1 | 1 ml x 1 | 4°C |
| Hoechst 33342 (200X) | 50 µl x 1 | 250 µl x 1 | -20 °C |
| BFA (250X) | Powder x 1 | Powder x 1 | -20 °C |
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## IV. MATERIALS REQUIRED BUT NOT PROVIDED

1. Standard fluorescence microscope
2. Calibrated, adjustable precision pipets, preferably with disposable plastic tips
3. Glass microscope slides
4. Glass cover slips
5. Deionized water
6. Growth medium
7. Paraformaldehyde
8. HBSS/PBS

## V. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use. The sample volumes below are sufficient for 96-wells 100 µl tests; adjust volumes as needed for the number of wells used in your experiment.

All unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

**C6 NBD Ceramide stock solution (100X)**

Briefly centrifuge prior to opening. Add 10 µL of Diluent into the vial of C6 NBD Ceramide and mix well. Store unused C6 NBD Ceramide stock solution at -20°C in single use aliquots. Avoid freeze-thaw cycles.

**Ceramide-BSA stock solution (20X)**

Briefly centrifuge the vial of C6 NBD Ceramide stock solution and BSA solution prior to opening. Add 400 µL of BSA solution into 5 µL of C6 NBD Ceramide stock solution, then add the mix in 100 µL Binding buffer immediately and vortex well for 1 min to generate Ceramide-BSA stock solution (20X). Store unused C6 NBD Ceramide stock solution at -20°C in single use aliquots. Avoid freeze-thaw cycles.

**Ceramide-BSA working solution**

For 1 test, add 5 µL of Ceramide-BSA stock solution (20X), 0.5 µL of Hoechst 33342 (200X) to 95 µL PBS to generate Ceramide-BSA working solution. Scale up as needed. Store unused Ceramide-BSA stock solution at -20°C in single use aliquots. Avoid freeze-thaw cycles.

**Optional:**

Hoechst 33342 can also be omitted from the Ceramide-BSA working solution. For fixed-cell staining, it is preferable to add Hoechst 33342 at the final step to 1X

concentration, incubate at RT for 5-10 min, and then observe under a fluorescence microscope

**BFA working solution (optional)**

Brefeldin A as Negative control caused loss of perinuclear Golgi-like NBD-ceramide labeling and produced a diffuse reticular/ER-like pattern. Briefly centrifuge the vial of BFA (250X) prior to opening. Add 1 mL of Diluent and vortex well to generate BFA working solution. Scale up as needed. Store unused BFA stock solution at -20°C in single use aliquots. Avoid freeze-thaw cycles.

**Back-exchange buffer (for Fixed Cells only)**

For 1 test, add 25 µL of BSA solution to 75 µL PBS to generate Back-exchange buffer. Scale up as needed. Store unused Back-exchange buffer at -20°C in single use aliquots. Avoid freeze-thaw cycles.

## VI. ASSAY PROCEDURE

1. Staining in Living Cells

1) Plate and treat cells as desired (such as 2 x $10^6$ cells/mL). Plate cells in a 96-well deep well microplate.

2) Golgi Disruption Positive Control treatment(optional): Add BFA working solution to the cell culture medium at a 1:200 dilution and incubate at 37 °C for 30-60 mins.

3) Treat Normal cells, Positive Control cells or other compounds processed cells using the same procedure. Remove the cell culture medium and wash with PBS once.

4) Remove PBS and add 100 µL/well of C6 NBD Ceramide working solution. Incubate at 4 °C for 30min in the dark.

5) Remove C6 NBD Ceramide working solution. Add 200 µL/well of medium and incubate at 37 °C for 30min in the dark.

6) Remove medium and wash with fresh medium 2-3 times.

7) Observe under a fluorescence microscope with FITC filter set.

2. Staining in Fixed Cells

1) Plate and treat cells as desired (such as 2 x $10^6$ cells/mL). Remove the cell culture medium and wash with HBSS/PBS twice.

2) Fix cells with 4% paraformaldehyde solution at room temperature for 5–10 minutes. Remove the fix solution and wash with HBSS/PBS twice.

3) Add 100 µL/well of C6 NBD Ceramide working solution. Incubate at 4 °C for 30min in the dark.

4) Remove C6 NBD Ceramide working solution and wash with HBSS/PBS once. Add 100 µL/well of Back-exchange buffer and incubate at room temperature for 30-90 min in the dark.

5) Remove Back-exchange buffer and wash with HBSS/PBS twice.

6) Optional: mount with aqueous mounting medium (e.g., Fluoromount-G).

7) Observe under a fluorescence microscope with FITC filter set.

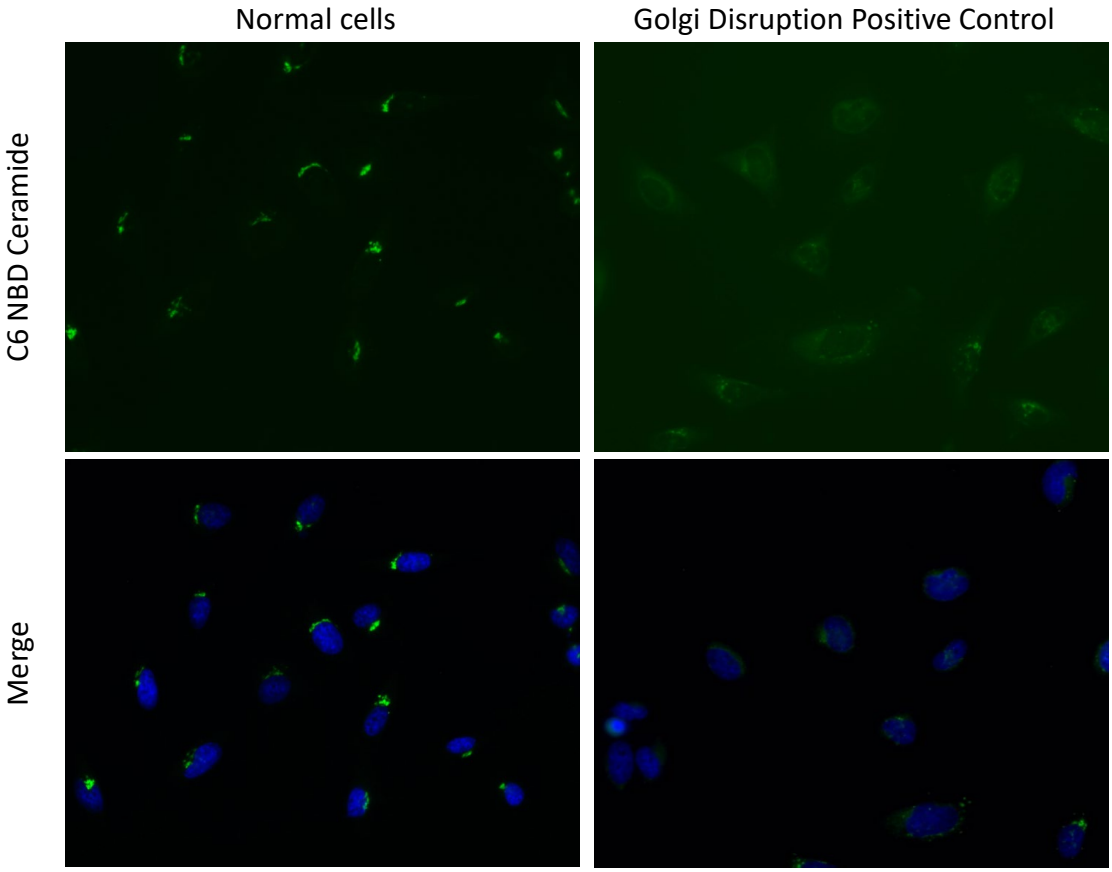
3. Fluorescence settings

Fluorescence microscope: Ex/Em = 488/525 nm

Recommended plate: Black wall/clear bottom

Instrument specification(s): FITC filter set

## VII. EXAMPLE DATA ANALYSIS AND FIGURES




Hela cells stained with Golgi Apparatus Staining Kit and analyzed on Confocal microscope using FITC filter set (Ex/Em = 488/525 nm) with 40× oil immersion objective

## VIII. STORAGE/STABILITY

The kit ships on wet ice and storage at 2-8 °C is recommended. Stored at -20°C for 1 year, avoid multiple freeze-thaw cycles.