Cell Navigator® NBD Ceramide Golgi Staining Kit *Green Fluorescence*

Catalog number: 22750
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 22750)
Component A: C6 NBD Ceramide	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Staining Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (25 mL)
Component C: DMSO	Freeze (< -15 °C)	1 vial (200 µL)
Component D: Hoechst 33342	Freeze (< -15 °C), Minimize light exposure	1 vial (50 µL)

OVERVIEW

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. This Cell Navigator® NBD Ceramide Golgi 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. This Cell Navigator® NBD Ceramide Golgi Staining Kit provides an optimized assay method for examining the morphology of the Golgi apparatus with a fluorescence microscope.

AT A GLANCE

Protocol Summary

1. Treat cells as desired
2. Add C6 NBD Ceramide working solution and incubate at room temperature or 37°C for 15~30 minutes
3. Replace with the Staining Buffer and incubate at room temperature or 37°C for 15~30 minutes
4. Observe under microscope using FITC filter set
5. Optional: Fix cells with 4% Formaldehyde

Important Note

Thaw one of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microscope

Emission	525 nm
Excitation	488 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	FITC filterset

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, 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)

Add 100 µL of DMSO (Component C) into the vial of C6 NBD Ceramide (Component A) and mix well.

Note Store unused C6 NBD Ceramide stock solution at -20^{o} C in single use aliquotes. Avoid freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

C6 NBD Ceramide working solution

Add 10 µL of NBD Ceramide stock solution (100X) to 990 µL Staining Buffer (Component B) to make NBD Ceramide working solution.

Optional: Add 10 µL Hoechst 33342 (Component D) to 1 mL NBD Ceramide working solution for nuclear stain. Observe under fluorescence microscope with DAPI filter set.

SAMPLE EXPERIMENTAL PROTOCOL

Staining protocol

1. Plate and treat cells as desired.
2. Add equal volume of C6 NBD Ceramide working solution directly in cell culture medium. **Note:** If you have cells in a 96 well plate with 100 µL/well cell culture medium then add 100 µL/well of C6 NBD Ceramide working solution.
3. Incubate at room temperature or 37 °C for 15~30min.
4. Remove the C6 NBD Ceramide working solution and replace with 100 µL/well of Staining Buffer (Component B).
5. Incubate at room temperature or 37 °C for 10~15 minutes.
6. Observe under a fluorescence microscope with FITC filter set.

Optional

Remove the staining buffer from Step 4, and add 100 µL/well/96-well plate of 4% formaldehyde fixative buffer (not supplied) to each well.
Note For non-adherent cells, add desired amount (such as 2X10^{6} cells/mL) of 4% formaldehyde fixative buffer.

Incubate plates for 20 to 30 minutes at room temperature. Remove fixative. Wash the cells with PBS 2-3 times, and replace with 100 µL/well of Staining Buffer (Component B).

Note The Cell Navigator™ NBD Ceramide Golgi Staining Kit also works well for aldehyde-fixed cells. After fixation, follow the staining protocol steps 2 to 6

EXAMPLE DATA ANALYSIS AND FIGURES

Placeholder for image details

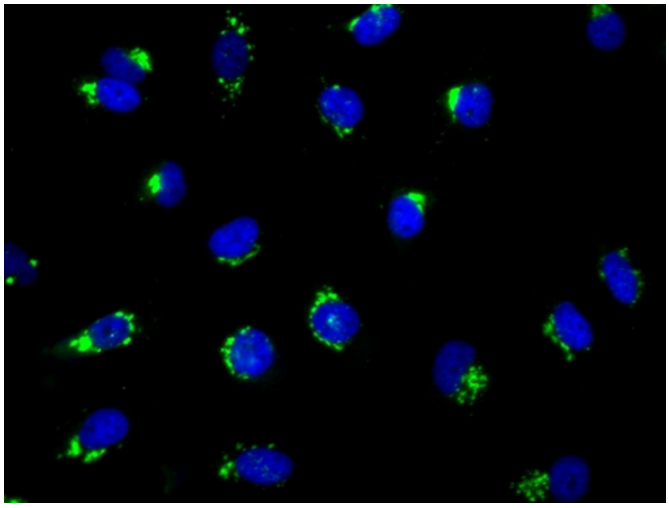

Figure 1. The fluorescence image of NBD Ceramide Golgi Staining in HeLa cells. Cells were stained with 100 µL of C6 NBD Ceramide working solution at 37 °C for 20min and followed with Hoechst 33342 stain. An intensely fluorescent threadlike structure, partially surround the nucleus, is identified as the Golgi apparatus.

DISCLAIMER

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