Cell Navigator® TMR Ceramide Golgi Staining Kit *Red Fluorescence*

Catalog number: 22752
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 22752)
Component A: GGR169-ceramide	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Staining Buffer	Freeze (< -15 °C)	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. Cell Navigator® TMR Ceramide Golgi Staining kit provides a simple and rapid way to stain Golgi in red fluorescence in live cells. Golgi apparatus is stained through the formation of the respective fluorescent metabolites. Cell Navigator® TMR Ceramide Golgi Staining Kit provides an optimized assay method for examining the morphology of the Golgi apparatus with a fluorescence microscope. In addition, GGR169-ceramide is pH-sensitive, thus uniquely serving as an excellent Golgi pH probe too.

AT A GLANCE

Protocol summary

1. Treat cells as desired
2. Add GGR169-ceramide working solution and incubate at room temperature or 37 °C for 15~30 minutes
3. Replace with the Staining Buffer
4. Observe under microscope using Cy3 filter set

Important

Thaw all the components at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microscope

Emission	Cy3/TRITC filter set
Excitation	Cy3/TRITC filter set
Recommended plate	Black wall/clear bottom

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

GGR169-ceramide stock solution (100X)

Add 100 µL of DMSO (Component C) to GGR-ceramide (Component A) to make GGR169-ceramide stock solution (100X).

Note Store the unused GGR-ceramide stock solution at -20 °C in single use aliquots to avoid freeze thaw cycles.

PREPARATION OF WORKING SOLUTION

GGR169-ceramide working solution

Add 10 µL of GGR169-ceramide stock solution (100X) to 990 µL of Staining Buffer (Component B) to make GGR169-ceramide working solution.

Optional: Add 10 µL of Hoechst 33342 (Component D) to 1 mL GGR169-ceramide working solution for nuclear stain. Observe under fluorescence microscope with DAPI filter set.

SAMPLE EXPERIMENTAL PROTOCOL

Following protocol should be used for the guidelines and can be modified as per requirements.

1. Plate and treat cells as desired.
2. Remove the cell culture medium. Optional: Cells can be washed with buffer of your choice.
3. Add 100 µL of GGR169-ceramide working solution directly into cell culture medium.
4. Incubate at room temperature or 37 °C for 15~30 minutes.
5. Remove the GGR169-ceramide working solution and wash once with DPBS or buffer of your choice.
6. Add 100 µL/well of Staining Buffer (Component B).
7. Observe under a fluorescence microscope with Cy3 filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

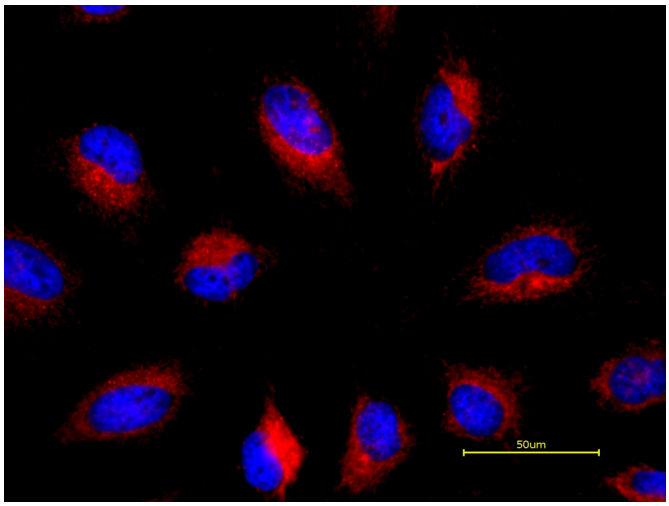

Figure 1. The representative fluorescence image of GGR169 Ceramide Golgi Staining in HeLa cells. Cells were stained with 100 µL of GGR169-ceramide working solution with Hoechst 33342 at 37 °C for 20 minutes. An intensely fluorescent thread like structure, partially surround the nucleus, is identified as the Golgi apparatus.

DISCLAIMER

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