

ab139483 –

**CytoPainter Golgi
Staining Kit – Green
Fluorescence**

Instructions for Use

Designed for the detection of Golgi bodies by
microscopy

This product is for research use only and is not
intended for diagnostic use.

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1. Introduction

ab139483 contains a Golgi apparatus-selective dye suitable for live cell, or aldehyde-fixed cell staining. Compared with other commercially available dyes for labeling Golgi bodies, Golgi Green dye is more faithfully localized to the target organelle, with minimal staining of the endoplasmic reticulum. Micromolar concentrations of dye are sufficient for staining mammalian cells, as validated with human cervical carcinoma cell line HeLa, human T-lymphocyte cell line, jurkat, canine kidney cell line MDCK, and Human bone osteosarcoma epithelial cell line U2OS.

One important application of Golgi Green dye is in fluorescence co-localization imaging with red fluorescent protein (RFP)-tagged proteins. This is a powerful approach for determining the targeting of molecules to intracellular compartments and for screening of their associations and interactions. Many organelle-targeting probes photo-bleach rapidly, are subject to quenching upon concentration in organelles, are highly toxic or only transiently associate with the target organelle, requiring imaging within a minute or two of dye addition. Consequently, Golgi Green dye, a new green-emitting, cell-permeable small molecule organic probe that spontaneously localizes to live or fixed Golgi apparatus, was developed. Golgi Green dye can be readily used in combination with other common UV and visible light excitable organic fluorescent dyes and various fluorescent proteins in multicolor imaging and detection applications. The dye emits in the FITC region of the visible light spectrum, and is

resistant to photo-bleaching, concentration quenching and photo-conversion. The kit should also be suitable for identifying Golgi body perturbing agents and thus can be a useful tool for examining retrograde flow mechanisms in cellular secretory pathways.

2. Product Overview

ab139483 has been specifically designed for use with RFP-expressing cell lines as well as cells expressing blue, cyan or orange fluorescent proteins (BFPs, CFPs, OFPs). Additionally, the kit is suitable for use with live or fixed cells in conjunction with probes, such as labeled antibodies, or other fluorescent conjugates displaying similar spectral properties as Texas Red, or coumarin. A blue nuclear counterstain, Hoechst 33342, is provided to highlight this organelle as well.

3. Assay Summary

Prepare reagents and allow warming to room temperature.



Wash cells with Assay Solution.



Pipette Dual Detection Solution to cells and incubate at 4°C for 30 minutes. Protect from light.



Wash cells with ice cold cell culture medium.



Incubate cells in medium for 30 minutes at 37°C.



Wash cells with Assay Solution.



Overlay cells with coverslip.



Analyze.

4. Components and Storage

A. Kit Contents

Item	Quantity	Storage Temperature
Golgi Green Detection Reagent	50 nmoles	$\leq -20^{\circ}\text{C}$
Hoechst 33342 Nuclear Stain	50 μl	$\leq -20^{\circ}\text{C}$
10X Assay Buffer 1	15 ml	$\leq -20^{\circ}\text{C}$
50X Assay Buffer 2	1.2 ml	$\leq -20^{\circ}\text{C}$

Reagents provided in the kit are sufficient for approximately 100 assays using live cells (adherent or in suspension).

B. Storage and Handling

Upon receipt, the kit should be stored upright at -80°C , protected from light. Avoid repeated freezing and thawing.

C. Additional Materials Required

- Standard fluorescence microscope.
- Calibrated, adjustable precision pipets, preferably with disposable plastic tips.
- Adjustable speed centrifuge with swinging buckets (for suspension cultures).
- Glass microscope slides.
- Glass cover slips.
- Deionized water.
- Anhydrous DMSO (optional).
- Growth medium (e.g. Dulbecco's Modified Eagle medium, D-MEM).
- Paraformaldehyde (optional, for fixation protocol).
- PBS (optional, for fixation protocol).

5. Pre-Assay Preparation

NOTE: Allow all reagents to thaw at room temperature before starting with the procedures. Upon thawing gently hand-mix or vortex the reagents prior to use to ensure a homogenous solution. Briefly centrifuge the vials at the time of first use, as well as for all subsequent uses, to gather the contents at the bottom of the tube.

1. 1X Assay Buffer

Allow the 10X Assay Buffer and the 50X Assay buffer to warm to room temperature. Make sure that the reagent is free of any crystallization before dilution. Prepare enough 1X Assay Buffer for the number of samples to be assayed For each 10 ml preparation of 1X Assay Solution, add 1ml 10X **Assay Buffer 1** and 0.2mL 50X **Assay Buffer 2** into 8.8ml deionized water. Mix well.

2. 100X Golgi Green Detection Reagent

Add 100 μ l of the 1X Assay Solution prepared above to the vial containing lyophilized Golgi Green Detection Reagent (50 nM). Vortex gently or slowly rotate the tube to dissolve.

3. Dual Detection Reagent

The concentration of the Golgi Green Detection Reagent for optimal staining will vary depending upon the application. Suggestions are provided to use as guidelines, though some modifications may be required depending upon the particular cell type employed and other factors such as the permeability of the

dye to the cells or tissues. To reduce potential artifacts from overloading the cells, the concentration of the dye should be kept as low as possible.

Prepare sufficient amount of Dual Detection Reagent for the number of samples to be assayed as follows: For every ml of 1X Assay Solution, add 10 μ l of the 100X Golgi Green Detection Reagent and 1 μ l of Hoechst 33342 Nuclear Stain.

NOTE:

- (a) The dyes may be combined into one staining solution or each may be used separately, if desired.
- (b) The Hoechst 33342 Nuclear Stain can be diluted further if its staining intensity is much stronger than the Golgi Green stain.
- (c) When staining BFP- or CFP-expressing cells, the Hoechst 33342 Nuclear Stain should be omitted due to its spectral overlap with these fluorescent proteins.

4. 4% Formaldehyde Solution

The following procedure is for preparation of 10 ml of 4% formaldehyde solution: Dilute 0.4 g of paraformaldehyde to a final volume of 10 mL with PBS. Mix well.

6. Assay Protocol

A. Staining Live Adherent Cells

1. Grow cells on coverslips inside a Petri dish filled with the appropriate culture medium. When the cells have reached the desired level of confluence, carefully remove the medium.
2. Wash the cells with 100 μ l 1X Assay Solution.
3. Dispense sufficient volume of Dual Detection Reagent to cover the monolayer cells (~100 μ l for cells grown on an 18 X 18 mm coverslip).
4. Protect samples from light and incubate for 30 minutes at 4°C.
5. Wash the cells 3 times using 100 μ l ice cold medium for each wash.
6. Incubate the cells in medium at 37°C for 30 minutes.
7. Wash the cells with 100 μ l 1X Assay Solution. Remove excess buffer and place coverslip on slide.
8. Analyze the stained cells by wide-field fluorescence or confocal microscopy (60X magnification recommended). Use a standard FITC filter set for imaging the Golgi bodies. Optionally, image the nucleus using a DAPI filter set and the RFP-tagged protein using a Texas Red filter set.

B. Staining live cells grown in suspension

1. Centrifuge cells for 5 minutes at 400 x g at room temperature (RT) to obtain a cell pellet.
2. Carefully remove the supernatant by aspiration and then wash the cells with 200 μ l of 1X Assay Solution.
3. Carefully remove the supernatant by aspiration and then add 100 μ l of Dual Detection Reagent to the cell pellet.
4. Protect samples from light and incubate for 30 minutes on ice.
5. Wash the cells 2 times using 200 μ l ice cold medium for each wash.
6. Re-suspend the cells in 100 μ l ice cold medium and then incubate the cells at 37°C for 30 minutes.
7. Wash the cells with 200 μ l 1X Assay Solution.
8. Re-suspend cells in 100 μ l 1X Assay Solution, then transfer the cells to a glass slide and overlay with a coverslip.
9. Analyze the stained cells by wide-field fluorescence or confocal microscopy (60X magnification recommended). Use a standard FITC filter set for imaging the Golgi bodies. Optionally, image the nucleus using a DAPI filter set and the RFP-tagged protein using a Texas Red filter set.

C. Staining aldehyde fixed cells

1. Grow cells on coverslips inside a Petri dish filled with the appropriate culture medium. When the cells have reached the desired level of confluence, carefully remove the medium.
2. Wash the cells with 100 μ l 1X Assay Solution.
3. Fix the cells for 10 minutes at 37°C with 4% formaldehyde solution.
4. Wash the cells 3 times using 100 μ l ice cold 1X Assay Solution for each wash.
5. Dispense sufficient volume of Dual Detection Reagent to cover the monolayer cells (~100 μ l for cells grown on an 18 X 18 mm coverslip).
6. Protect samples from light and incubate for 30 minutes at 4°C.
7. Wash the cells 3 times using 100 μ l 1X Assay Solution for each wash.
8. Add 100 μ l fresh medium to cover the cells and allow to incubate at room temperature for 45 minutes.
9. Wash the cells with 100 μ l 1X Assay Buffer. Remove excess buffer and place coverslip on slide.

10. Analyze the stained cells by wide-field fluorescence or confocal microscopy (60X magnification recommended). Use a standard FITC filter set for imaging the Golgi bodies. Optionally, image the nucleus using a DAPI filter set and the RFP-tagged protein using a Texas Red filter set.

NOTE: Avoid the use of detergents and ethanol/methanol/acetone fixatives.

7. Data Analysis

A. Filter set selection

The selection of optimal filter sets for a fluorescence microscopy application requires matching the optical filter specifications to the spectral characteristics of the dyes employed in the analysis. Consult the microscope or filter set manufacturer for assistance in selecting optimal filter sets for your microscope.

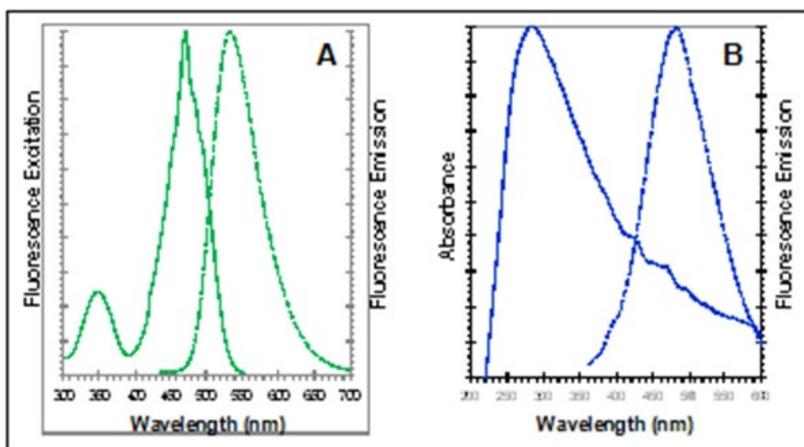


Figure 1. Fluorescence excitation and emission spectra for Golgi Green dye (panel A) and absorbance and fluorescent emission spectra for Hoechst 33342 dye (panel B). All spectra were determined in 1X Assay Solution.

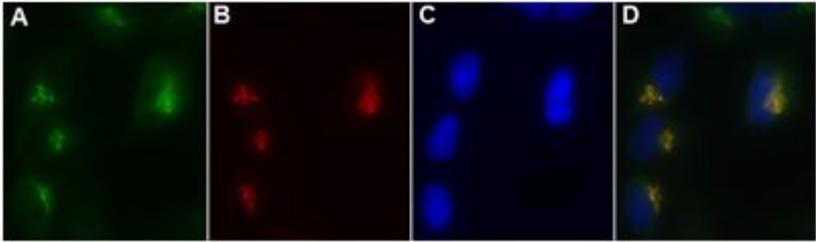


Figure 2. Golgi Green dye co-localizes with N-acetylgalactosaminyltransferase-2, fused to red fluorescent protein (RFP). (A) Golgi Green dye, (B) HeLa cells expressing RFP-N-acetylgalactosaminyltransferase-2, (C) Hoechst 33342 staining, (D) composite image.

B. Results

The Golgi apparatus is an organelle found in eukaryotic cells, responsible for processing and packaging proteins and lipids during their subcellular sorting. One prominent post-translational modification proteins and lipids undergo within the Golgi apparatus is glycosylation through the actions of various glycosidases and transferases. Interfering with Golgi apparatus function by, for example, treatment with certain drugs, typically leads to perturbation of Golgi function, including blocking of secretion, altering morphology, dispersion of Golgi structural elements, loss of cisternal stacks or inhibition of vesicular transport.

ab139483 provides a rapid and convenient method for visualizing the Golgi body within living cells, without a requirement for lengthy transfection procedures. In addition to serving as a live cell permeable dye, Golgi Green detection reagent is also localized within the Golgi body of fixed cells. Upon staining with the dual detection reagent, the nucleus should fluoresce blue, as detected with a DAPI filter set, and the Golgi apparatus should exhibit prominent green fluorescence, appearing as a perinuclear reticular network within the cell, when employing a FITC filter set. Only minor staining of other membranes within the cell should be observed.

8. Troubleshooting

Problem	Reason	Solution
Golgi apparatus is not sufficiently stained.	Very low concentration of Golgi Green detection reagent was used or cells were incubated too long after labeling.	Either increase the labeling concentration or limit the time allowed for the cells to grow after the dye has been removed. We recommend labeling for 30 minutes and incubating cells for 30 minutes after the label has been removed.
Precipitate is seen in the 10X Assay Buffer 1.	Precipitate forms at low temperatures.	Allow solution to warm to room temperature or 37°C, then vortex to dissolve all precipitate.

<p>The blue nuclear counterstain is too bright compared to the green Golgi stain</p>	<p>Different microscopes, cameras and filters may make some signals appear very bright.</p>	<p>Reduce the concentration of the nuclear counterstain or shorten the exposure time.</p>
<p>The green Golgi dye appears to stain more than just the Golgi bodies.</p>	<p>Excess Golgi Green dye was used or cells were not washed well enough after staining with medium containing serum</p>	<p>Reduce the concentration of Golgi green detection reagent and/or add additional washes with ice cold medium (containing 10% serum) and extend the incubation time in medium after staining.</p>

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