Product Manual

Organelle-ID RGB® III Assay Kit

for detection of the Golgi apparatus, endoplasmic reticulum and nucleus by microscopy

Catalog #: ENZ-51032-K100

100 assays

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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

TABLE OF CONTENTS

INTRODUCTION

The endoplasmic reticulum (ER) and Golgi apparatus (GA) are the primarily organelles responsible for the proper sorting of lipids and proteins within cells.[1] Direct membrane continuity between these organelles has been established using different analytical methods, ranging from reconstruction of serial sections in transmission electron microscopy to functional analysis of the transport process itself. After synthesis, folding and quality control, the lipid and protein cargo exits from the ER and enters the GA through the ER-Golgi interface. Within the ER-Golgi interface, COPII-mediated concentration of membrane and soluble cargo occurs and various post-translational modifications take place prior to delivery to the GA, including O-glycosylation, acylation, palmitoylation and mannose-6-phosphate attachment (lysosomal targeting signal). Transient ER-Golgi connections are likely to serve a role in the diffusion of cargo proteins as well as the recycling of organelle-resident proteins. The structure and functions of the various compartments along the secretory pathway are considered complicated and tools for the simple visualization and unambiguous categorization of the ER and GA in living cells have been lacking.

Enzo Life Sciences' Organelle-ID RGB® III Assay Kit contains GA-selective, ER-selective and nucleus-selective dyes suitable for live cell staining. Compared with other commercially available dyes for labeling Golgi bodies, the green dye component of the Organelle-ID RGB® III Detection Reagent is more faithfully localized to the Golgi apparatus, with minimal staining of the endoplasmic reticulum.[2] The red dye component of the reagent stains the endoplasmic reticulum with high fidelity and is specifically designed for use with green fluorescing probes. Organelle-ID RGB® III Assay Kit is 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.

The kit should also be suitable for identifying Golgi body and endoplasmic reticulum perturbing agents and thus can be a useful tool for examining the transport and recycling of molecules from the GA to ER in cellular secretory pathways.[3]

SAFETY WARNINGS & PRECAUTIONS

Handle with care

Avoid freeze / thaw cycles

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

- This product is for research use only and is not intended for diagnostic purposes.
- Some components of this kit may contain hazardous substances. Reagents can be harmful if ingested or absorbed through the skin and may cause irritation to the eyes. They should be treated as possible mutagens, should be handled with care and disposed of properly.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas. All blood components and biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.
- To avoid photobleaching, perform all manipulations in low light environments or protected from light by other means.

REAGANTS SUPPLIED AND STORAGE

All reagents are shipped on dry ice. Upon receipt, the kit should be stored upright and protected from light at ≤-20°C. When stored properly, these reagents are stable for at least twelve months. **Avoid repeated freezing and thawing**.

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

Reagent	Quantity
Organelle-ID RGB® Reagent III (lyophilized)	1 vial
10X Assay Buffer 1	15ml
50X Assay Buffer 2	1.2ml

OTHER MATERIALS NEEDED

1. Standard fluorescence microscope
2. Calibrated, adjustable precision pipets, preferably with disposable plastic tips
3. Adjustable speed centrifuge with swinging buckets (for suspension cultures)
4. Glass microscope slides
5. Glass cover slips (18 x 18 mm)
6. Deionized water
7. Anhydrous DMSO (optional)
8. Growth medium (e.g. Dulbecco's Modified Eagle medium, D-MEM)

METHODS AND PROCEDURES

***NOTE**: PLEASE READ THE ENTIRE PROCEDURE BEFORE STARTING. 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.*

REAGENT PREPARATION

1. 1X Assay Solution

Allow the 10X Assay Buffer 1 and the 50X Assay Buffer 2 to warm to room temperature. Make sure that the reagents are free of any crystallization before use. Prepare enough 1X Assay Solution for the number of samples to be assayed. For each 10ml 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. Organelle-ID® RGB Reagent III Solutions

a. **100X Organelle-ID RGB® Reagent III**. Add 100µl of freshly prepared 1X Assay Solution (from step A-1) to the vial containing lyophilized Organelle-ID RGB® Reagent III. Vortex gently or slowly rotate the tube to dissolve. This re-suspended reagent may be stored at –20°C for up to 3 months.

b. **1X Organelle-ID RGB® Reagent III**. Add 10µl of 100X Organelle-ID RGB® Reagent III solution (from step 2a, above) per 1ml of freshly prepared 1X Assay Solution (from step A-1). Mix well.

***NOTE**: The dilution of the Organelle-ID RGB® Reagent III provided in this procedure is only a suggestion. The concentration of Organelle-ID RGB® Reagent III for optimal staining will vary depending upon the application, the cell type employed and other factors such as the permeability of the dye to the cells or tissues. To reduce potential artifacts from overloading of the cells, the concentration of the dye should be kept as low as possible.*

STAINING LIVE, ADHERENT CELLS

1. Grow cells on 18 x 18 mm coverslips, or tissue culture treated slides, 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 100µl 1X Assay Solution (from item 1 on previous page).
3. Dispense 100µl of 1X Organelle-ID RGB® Reagent III (see step 2b in previous section) to cover the monolayer.
4. Protect samples from light and incubate for 30 minutes at 4°C.
5. Wash the cells 3 times using 100µl **ice cold** medium for each wash.
6. Add fresh ice cold medium and incubate the cells at 37°C for 30 minutes.
7. Wash the cells with 100µl 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, a DAPI filter set for the nucleus and a Texas Red filter set for the endoplasmic reticulum.

STAINING LIVE CELLS GROWN IN SUSPENSION

1. After growth, 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 200µl of 1X Assay Solution (from item 1 on previous page).
3. Carefully remove the supernatant by aspiration and then add 100µl of 1X Organelle-ID RGB® Reagent III (see item 3 in previous section) to the cell pellet.
4. Protect samples from light and incubate for 30 minutes on ice.
5. Wash the cells 2 times using 200µl ice cold medium for each wash.

6. Re-suspend the cells in 100µl ice cold medium and then incubate the cells at 37°C for 30 minutes.

7. Wash the cells with 200µl 1X Assay Solution.

8. Re-suspend cells in 100µl 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, a DAPI filter set for the Nucleus and a Texas Red filter set for the endoplasmic reticulum.

APPENDICES

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 (see Figure 1 for the spectra of the dyes). Consult the microscope or filter set manufacturer for assistance in selecting optimal filter sets for your microscope

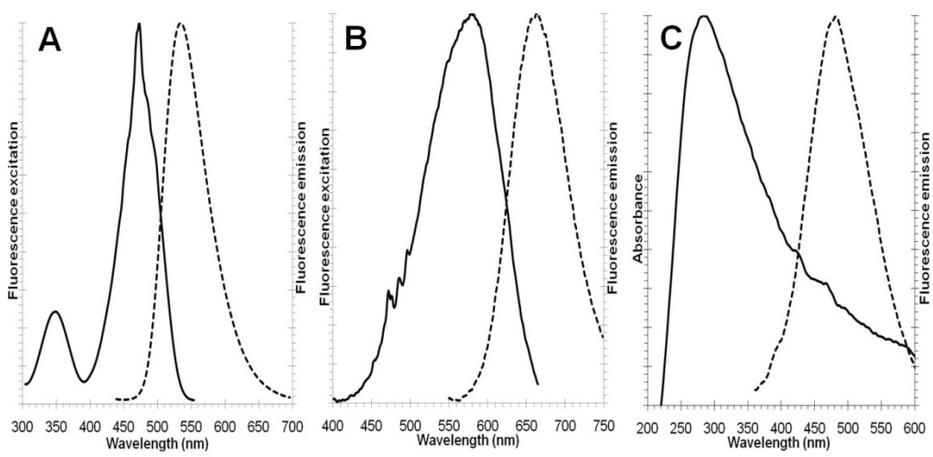

Figure 1. Fluorescence excitation and emission spectra for the green dye (panel A), red dye (panel B) and absorbance and fluorescent emission spectra for the blue dye (panel C). All spectra were determined in 1X Assay Solution.

RESULTS

The Golgi apparatus and endoplasmic reticulum are organelles found in eukaryotic cells, responsible for processing and packaging proteins and lipids during their subcellular sorting.[1] One prominent post-translational modification proteins and lipids undergo within the Golgi apparatus is glycosylation through the actions of various glycosyl transferases and glycosidases. Interfering with endoplasmic reticulum or Golgi apparatus function by, for example, treatment with certain drugs, typically leads to perturbation of function, including blocking of secretion, altering morphology, dispersion of structural elements, loss of Golgi cisternal stacks or inhibition of vesicular transport.[2]

The Organelle-ID RGB® III Assay Kit provides a rapid and convenient method for visualizing the Golgi body and endoplasmic reticulum within living cells, without a requirement for lengthy transfection procedures. Upon staining with the Organelle-ID RGB® Reagent III, the nucleus should fluoresce blue, as detected with a DAPI filter set. The Golgi apparatus should exhibit prominent green fluorescence, appearing as a perinuclear reticular network within the cell, when employing a FITC filter set. The endoplasmic reticulum should fluoresce red using a Texas Red filter set. Only minor staining of other membranes within the cell should be observed.

REFERENCES

1. Mironov, A. and Pavelka, M. (2008) “The Golgi apparatus as a crossroads in intracellular traffic” In: The Golgi Apparatus: State of the art 110 years after Camillo Golgi’s Discovery. (Mironov and Paveka, Eds) Springer, New York ISBN: 978-3-211-76309-4
2. Deng Y, Bennink JR, Kang HC, Haugland RP, Yewdell JW. Fluorescent conjugates of brefeldin A selectively stain the endoplasmic reticulum and Golgi complex of living cells. J Histochem Cytochem. 1995 Sep;43(9):907-15.
3. Dinter A and Berger EG Golgi-disturbing agents. Histochem Cell Biol. 1998 May-Jun;109(5-6):571-90.

TROUBLESHOOTING GUIDE

Problem	Potential Cause	Suggestion
Organelles not sufficiently stained.	Very low concentration of Organelle-ID RGB® III dye 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.
The blue nuclear counterstain is too bright compared to the green Golgi stain.	Different microscopes, cameras and filters may make some signals appear very bright.	Shorten the exposure time.
The green Golgi dye appears to stain more than just the Golgi bodies.	Excess Golgi green dye was used or cells were not washed well enough after staining with medium containing serum.	Reduce the concentration of Organelle-ID RGB® III Detection Reagent and/or add additional washes with ice cold medium (containing 10% serum) and extend the incubation time in medium after staining.

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