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ab189819 Membrane Fluidity Kit

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For the detection of membrane fluidity in cells

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

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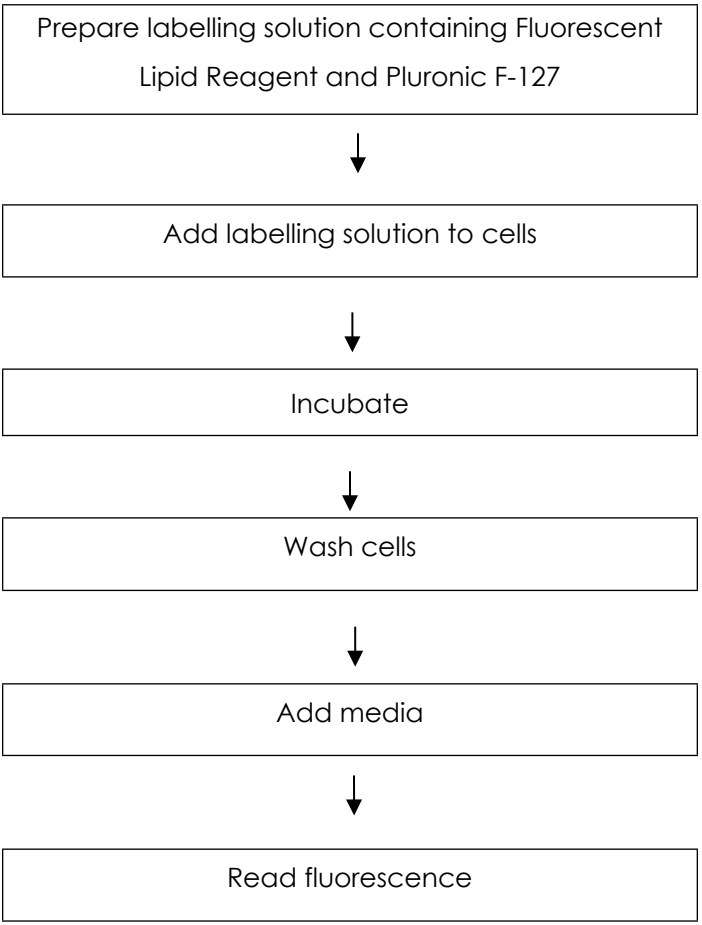
1. Overview

The dynamic properties of the cell membrane and cytoplasmic microtubules and microfilaments, as well as the dynamic movement of lipids in micelles and vesicles is of importance in such diverse areas as activation of polymorphonuclear leukocytes and chemotaxis, activation of membrane enzyme systems and the specific assembly or mobilization of microtubules and microfilaments, enhancement of the affinity of chemoattractant receptors, as well as being associated with a variety of pathological syndromes related to membrane fluidity.

It has been recognized that the rotational mobility of fluorescent or magnetic resonant probes is different from that observed in lateral diffusion. Membrane fluidity or "membrane viscosity" for short range lateral diffusion has best been measured using lipid analog probes that, when interacting, exhibit changes in their spectral properties. One of the best systems for use in such studies are lipophilic pyrene probes, such as pyrenedecanoic acid (PDA), that undergo transient dimerization upon spatial interaction. When cells are treated with PDA, the fluorescent probe incorporates into the cell's plasma membrane through the fatty acid moiety. As the probe diffuses through the membrane it forms homodimers, or

"excimers", with the rate of excimer formation proportional to the membrane fluidity. When excimers form, the emission spectrum of the pyrene probe shifts dramatically to the red (longer wavelength), and by measuring the ratio of excimer (Em ~470nm) to monomer (Em max. ~400nm) fluorescence, a quantitative monitoring of the membrane fluidity can be attained. These measurements can provide kinetic information, as well as *in vivo* monitoring of cellular function by both flow cytometry and microscopic analysis.

2. Protocol Summary



3. Materials Supplied and Storage

Fluorescent reagents and fluorescent labeling solutions or samples should be handled with care; keep on ice when not in use, and store at 4°C. Reagents should be stable for at least 6 months following purchase. Unstable background fluorescence readings for the Lipid Reagent in cell-free samples will indicate decomposition. In case of contact with skin or eyes, wash thoroughly with soap and cold water. These materials are intended for research purposes only. Use in drug or manufacturing processes is strictly prohibited. Please contact us for information on use or licensing.

Item	Quantity	Storage Temperature
Fluorescent Lipid Reagent	2 mL	4°C
Pluronic F-127	50 mg	4°C
Perfusion Buffer	25 mL	4°C
Reference Standard	2 mL	4°C

4. Materials Required, Not Supplied

- 96-well microplates
- Fluorescence microplate reader
- CO_2 Incubator
- Flow Cytometer

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

6. Reagent Preparation

6.1. **Fluorescent Lipid Reagent:** 2 mL of 100 µM pyrenedecanoic acid (PDA) in ethanol. Dilute with Perfusion Buffer (adherent cells) or media (non-adherent cells) to prepare the reagent for use in the Assay protocol.

6.2. **Pluronic F127**: To ensure efficient labeling, it is recommended that Pluronic F127 (50 mg provided) be added to the labeling solution. To use: Prepare a 1% stock solution of by diluting 50 mg Pluronic F127 in 5 mL deionized water.

6.3. **Reference Standard:** 2 mL of 2 mM pyrene in dimethyl sulfoxide (DMSO). Dilute with ethanol, water, buffer or media to the appropriate concentration for spectrometer calibration. ***note that the Reference Standard is only necessary if you would like to calibrate the wavelengths of monomer and excimer fluorescence in your experimental buffer. It is not used in the experimental protocol.**

7. Assay Protocol

Note: For adherent cells grown in culture, use the Perfusion Buffer provided. For non-adherent cells, prepare cells in media (for example Dulbecco's modified Eagle's medium) containing 10% fetal bovine serum, penicillin (100 units/mL) and streptomycin (100 µg/mL).

7.1. Plate adherent cells or cells in suspension in a multi-well plate. Alternatively, cells can be plated on chamber slides for live cell imaging.

Note: The ratio of excimer to monomer fluorescence should be independent of cell number if the staining levels for all samples are kept consistent.

7.2. Prepare labeling solution containing 5 µM* Fluorescent Lipid Reagent with either Perfusion Buffer (for adherent cells) or media (for cells in suspension). Supplement the labeling solution with Pluronic F127 at a final concentration of .08% (add 87 µL 1% Pluronic F127 stock solution for every 1 mL labeling solution)

7.3. Incubate cells with Fluorescent Lipid Reagent/Pluronic F127 solution for 1 hour at 25°C in the dark. For cells in suspension, gentle agitation is recommended to ensure the reaction system is homogenous.

Note: It is recommended that measurements be made in duplicate wells at minimum. Since excimer formation is time dependent, a time course for the experiments may be beneficial for initial trials.

7.4. Remove the unincorporated PDA by washing cells twice with either perfusion buffer or media.

7.5. Resuspend or bathe the cells in media.

7.6. Store unread plates (appropriately covered with sterile parafilm or plastic wrap) at 25°C or in the incubator if they are not to be read immediately.

7.7. Monomer and excimer fluorescence can be analyzed by one of the following methods; The user is asked to

consult with the manufacturer (or instrument manual) of the instrument in use for appropriate filter set(s) needed for monitoring at the indicated wavelengths. If applicable, the provided Reference Standard can be used to optimize these spectrometer conditions.

7.7.1. Fluorescence microplate reader (**recommended**): Read fluorescence at both 400nm and 450-470nm using the appropriate filter for excitation at 350nm. Subtract the blank(s) from each sample, average the readings of duplicates, and calculate the normalized excimer to monomer fluorescence ratio (Ie/Im)

7.7.2. Live-cell Fluorescence Microscopy: Typical epifluorescence microscopic analysis is performed using excitation light filtered by a 350-nm or 370-mm dichroic filter. Cell images of emitted light should be taken utilizing a 405-nm filter for monomer fluorescence and a 470-nm filter for excimer fluorescence. Use care when changing filters to avoid disturbing the cells and optical path. Cells are best viewed on a 40X lens. Background images at the monomer and excimer emission wavelengths should be obtained with unlabeled cells before labeling *in situ* in the microscope chamber, and background values at each emission wavelength should be subtracted from images of dye-loaded cells. Acquisition of individual images may require up to 5 seconds of image averaging. Ratio images of the fluorescence at the excimer-to-monomer emission wavelengths can then be calculated pixel-by-pixel. (When necessary, images should be displaced in the x and y directions by an integral number of pixels to align monomer and excimer images, as slight misalignment of images may occur when emission filters are changed.)

7.7.3. Flow Cytometry: FACS instruments are typically equipped with bandpass filter of 400 and 450nm (70nm bandwidth) for monitoring monomer and excimer fluorescence respectively. An argon ion laser (360nm emission, 20 mW output) may be used for excitation. Subtract blank/background fluorescence levels and calculate Ie/Im ratio as before.

*Previous studies have shown that the labeling of cells is virtually independent of the initial PDA concentration within the range of 1-25 µM. However, the working range of the assay can be determined for individual experimental conditions. If this is preferred, a calibration curve can be generated by plotting the normalized fluorescence ratio (Ie/Im) vs. PDA concentration to determine the optimal PDA concentration for further experiments. Additionally, membrane fluidizers (such as aliphatic alcohols) can be used as positive controls to increase Ie/Im ratio.

8. Typical Data

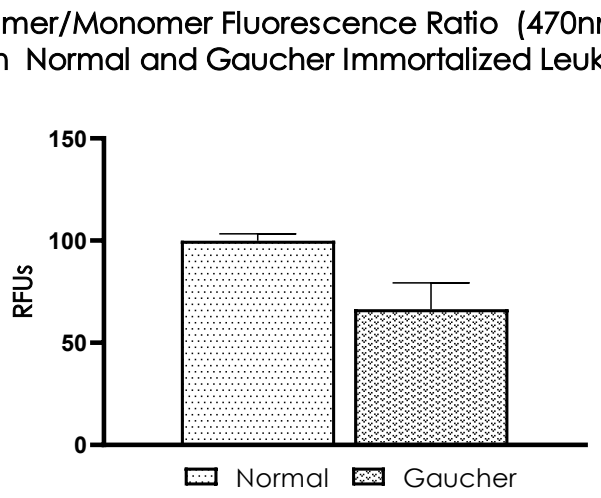


Figure 1: Gaucher Disease leukocytes exhibit decreased membrane fluidity relative to healthy controls Immortalized leukocytes were incubated for 1 hour incubation with 2 µM PDA and .08% pluronic F-127. PDA fluorescence was measured by exciting at 350nM and taking emission values at 400nM (monomer) and 470nM (excimer). Relative membrane fluidity is a ratio of excimer to monomer fluorescence. Previous studies have demonstrated that cells isolated from Gaucher Disease patients exhibit decreased membrane fluidity.

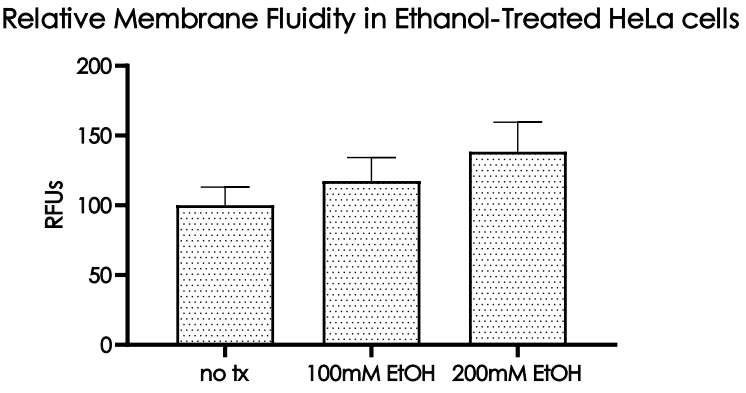


Figure 2: Ethanol treatment increases membrane fluidity in HeLa cells HeLa cells were treated with 100mM or 200 mM Ethanol for 48 hours, followed by 1 hour incubation with 2 µM PDA and .08% pluronic F-127. PDA fluorescence was monitored by exciting at 350 nM and taking emission values at 400nM (monomer) and 470nM (excimer). Relative membrane fluidity is a ratio of excimer to monomer fluorescence. Aliphatic alcohols such as EtOH are known to increase membrane fluidity.

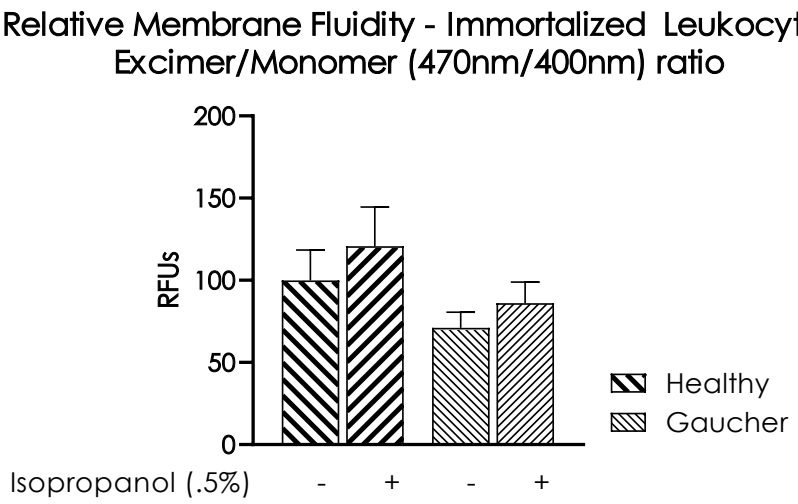


Figure 3: Isopropanol treatment increases membrane fluidity in primary leukocytes Primary leukocytes were treated with .5% isopropanol for 1 hour, followed by 1 hour incubation with 5 µM PDA and .08% pluronic F-127. PDA fluorescence was measured by exciting at 350 nM and taking emission values at 400nM (monomer) and 470nM (excimer). Relative membrane fluidity is a ratio of excimer to monomer fluorescence.

9. Troubleshooting

1. For measuring membrane fluidity in cells in suspension, such as blood cells, it may be difficult to keep cell counts consistent across treatment conditions, especially following multiple washes. Will cell number influence the resulting Ie/Im across treatment conditions?

   Since the readout for this experiment is a ratio of excimer-to-monomer fluorescence, these values should be independent of cell number so long as staining levels for all samples are kept consistent. You should have a wide range of labeling concentrations to work in, without serious error, as previous studies have shown that the labelling of cells is virtually independent of the initial fluorescent probe concentration in the range of 1-25 µM. If you would still like to optimize the assay, creating a standard fluorescence curve may be of value. Prior to running your experiment, this standard curve can be produced by treating cells (at the cell count that will be used in your experiment, or at multiple cell counts) with PDA at a range of concentrations between 1-25 µM. (For treating a large number of cells in suspension, higher concentrations of PDA can be tested.) Use a PDA concentration and cell count for your

experiments that is within the dynamic range of your standard curve.

2. How should one determine background fluorescence?

   Background fluorescence should be determined in wells with untreated cells. Additionally, if you would like to calibrate your spectrophotometer to optimize the emission wavelengths of monomer and excimers in your specific assay buffer, a 2 mM pyrene solution is included in the kit for use as a reference standard. The resulting spectra can be used to optimize emission maxima for specific experimental conditions. This standard can be additionally be used at your treatment concentration to establish an upper limit for fluorescence, provided this fluorescence is below saturation.

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

10. Notes

Technical Support

For all technical or commercial enquiries please go to:

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www.abcam.co.jp/contactus (Japan)