ab189819

Membrane fluidity kit

Instructions for Use

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. Introduction

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 the lipophilic pyrene probes that undergo eximer formation upon spatial interaction. When eximers form, the emission spectrum of the pyrene probe shifts dramatically to the red (longer wavelength). By measuring the ratio of monomer (Em max. 372nm) to eximer (Em 470nm) 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

Prepare labeling solution containing Fluorescent Lipid Reagent and Pluronic F-127

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Add labeling solution to cells

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Incubate

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Wash cells

↓

Add media
3. Kit Contents

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescent Lipid Reagent</td>
<td>2 mL</td>
<td>Store cold 4°C</td>
</tr>
<tr>
<td>Reference Standard</td>
<td>2 mL</td>
<td>Store at room temperature</td>
</tr>
<tr>
<td>Perfusion Buffer</td>
<td>25 mL</td>
<td>Store cold 4°C</td>
</tr>
<tr>
<td>Pluronic F-127</td>
<td>50 mg</td>
<td>Store at room temperature</td>
</tr>
</tbody>
</table>
4. Storage and Handling

Fluorescent reagents and fluorescent labeling solutions or samples should be handled with care, kept cold (ice-bath) when not in use, and can be stored frozen (-20°C). In case of contact with skin or eyes wash thoroughly with soap and cold water. Reagents should be stable for at least 6 months following purchase. Unstable background fluorescence readings for blank samples will indicate decomposition. 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.

5. Additional Materials Required

- 96-well microplates
- Fluorescence microplate reader
- CO₂ Incubator
- Flow Cytometer
6. Reagent Preparation

- **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.

- **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.
7. Assay Protocol

Note: For adherent cells grown in culture, use Perfusion Buffer. 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).

It is recommended that measurements be made in duplicate, if possible, and that the approximate concentration range of the fluorescent probe be adjusted for optimum signal and sensitivity. Previous studies have indicated that the labelling of cells is virtually independent of the initial fluorescent probe concentration in the range of about 1- 25 µM. Since eximer formation will be time dependent, a time course for the experiments should also be generated for initial trials. The emission of the highly fluorescent eximer is monitored at 445 - 470nm using excitation at 350nm. The user is asked to consult with the manufacturer (or instrument manual) for the particular instrument in use for appropriate filter set(s) needed for monitoring at these wavelengths. Typical epifluorescence microscopic analysis is performed using an excitation filter (350nm dichroic filter), a dichroic filter (370nm) and emission filters for monomer fluorescence (405nm interference filter, 10 nm bandwidth) and eximer fluorescence (470nm cut-on filter). For flow cytometric analysis, the FACS instrument is typically equipped with bandpass filter of 400 and 450nm (70nm bandwidth) for monitoring monomer and eximer fluorescence respectively. An
argon ion laser (360nm emission, 20 mW output) may be used for excitation.

To normalize data, each cell suspension or plate is monitored at exactly the same time (20 min.) after equilibration with the probe. The eximer-forming rate is dependent on the concentration of incorporated probe in the cell membrane, but with concentrations of the probe above approximately 2 µM, the ratio of eximer/monomer is typically independent of initial concentration. The working range of the assay will need to be determined for each individual experiment. Adjust working concentrations accordingly.

- 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 Pluronic F127 in deionized water. Prepare labeling solution below to give a final concentration of 0.08%.

- Prepare labeling solution containing 10-20 µM Fluorescent Lipid Reagent and a final concentration of 0.08% Pluronic F127 with either Perfusion Buffer (for adherent cells) or media (for cells in suspension).
• Incubate/mix the sample of cells in culture or cell suspension with labelling solution for 20 minutes at 25°C in the dark, with rotation for cell suspensions, to make sure the reaction system is homogeneous.

• Remove the unincorporated pyrenedecanoic acid by two washes of the cells with either perfusion buffer or media.

• Resuspend or bathe cells in media.

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

• Read fluorescence at both 400 and 450-470nm using the appropriate laser emission or excitation filter for excitation at 360nm. Use Reference Standard for optimizing spectrometer conditions, if applicable. Dilute reference sample with water, buffer or media as necessary to bring the concentration to similar levels as those obtained in the sample system.

• Subtract fluorescence from blank(s) from each sample. Average the readings of duplicate samples. Calculate the normalized eximer to monomer fluorescence ratio (Ie/Im).

• Generate a calibration curve by plotting the normalized* fluorescence ratio (Ie/Im) vs. PDA concentration (log-log).
Using the calibration curve from above, determine the concentration (range) of PDA to be used in labeling experiments and use concentration in all further experiments to determine the kinetics of membrane fluidity in the cells/tissue suspension, as well as the effect of various other added membrane fluidizers (i.e. aliphatic alcohols, etc.) on the Ie/Im ratio.
8. Troubleshooting

1. We're trying to use the kit to measure the membrane fluidity of blood cells in conjunction with a fluorescence spectrophotometer. But it's difficult to control the cell number of each sample exactly at the same level, especially after twice washing with PBS. So I am wondering whether the cell number will influence the result if we use the ratio of le/lm?

   The ratio of eximer to monomer fluorescence should be independent of cell number, as long as the staining levels for all samples are consistent and about the same. Also, please note that "previous studies have shown that the labelling of cells is virtually independent of the initial fluorescent probe concentration as long as the range is about 1 -25 µM". So you should have a pretty wide range of labeling concentrations to work in, without serious error.

2. Another question is about the blank. Should we use cell suspension without PDA, or PDA without cells, as a blank?

   We usually just run the PDA as the blank, but I would suggest running both blanks if you have some wells, as you could then subtract any endogenous fluorescence from your samples.
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).
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