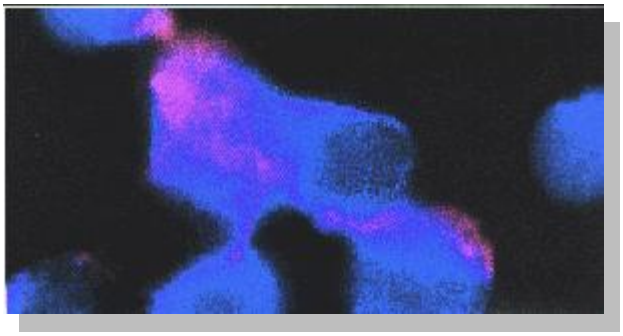
Product Information Sheet



MEMBRANE FLUIDITY KIT
(Product M0271)

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Membrane Fluidity Kit
(Product M0271)

NOTE: The following information is given as a viable methodology for use of the MarkerGene™ Membrane Fluidity Kit. The user may determine their own best conditions for use dependant on the specific conditions present in their experiment.

I. 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 [1], activation of membrane enzyme systems and the specific assembly or mobilization of microtubules and microfilaments [12], enhancement of the affinity of chemoattractant receptors [3], as well as being associated with a variety of pathological syndromes related to membrane fluidity [4-6].

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. 372 nm) to eximer (EM 470 nm) 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 [7] and microscopic [8] analysis.

II. MATERIALS

A.) 1 **Fluorescent Lipid Reagent**. 2.0 mL of 100 μM pyrenedecanoic acid (PDA) in 0. 1 M phosphate buffer @ pH 7.4). Dilute with buffer or media to prepare the reagent for use in step 1 below.

B.) 2 **Reference Standard**. 2.0 mL of 2 mM pyrene in dimethylsulfoxide. Dilute with ethanol, water, buffer or media to the appropriate concentration (i.e. 1/1000 for a 2 μM solution) for spectrometer calibration.

C.) Buffer Solutions/Media. (1 x 25 mL perfusion buffer 3 provided) Prepare buffer solutions as is common for the cells or experiment in progress or as suggested below. For adherent cells grown in culture, use perfusion buffer 3, containing 100 mM sodium chloride in 5 mM Hepes/Tris buffer, pH 7.4 with 2 mM $MgCl_2$, 5 mM potassium chloride, 2 mM calcium chloride, 5 mM glucose and 50 mM mannitol. For cells in suspension, prepare cells in media (for example Dulbecco's modified Eagle's medium) containing 10% fetal bovine serum, penicillin (100 units/mi) and streptomycin (100 μg/mL).

D.) 4 **Pluronic F127**, 50mg. Can be used to increase labeling. Prepare a 0.08% solution in deionized water.

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 (-20ºC). 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.

III. MEMBRANE FLUIDITY ASSAY

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 labeling of cells is virtually independent of the initial fluorescent probe concentration in the rage of about 1- 25 μM. Since eximer formation will be time dependant, a time course for the experiments should also be generated for initial trials. The emission of the highly fluorescent eximer is monitored at 445 - 470 nm using excitation at 350 nm. 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 (350 nm dichroic filter, Omega Optical Co., Brattleboro, VT), a dichroic filter (370 nm) and emission filters for monomer fluorescence (405 nm interference ftlter, 10 nm bandwidth) and eximer fluorescence (470 nm cut-on filter). For flow cytometric analysis, the FACS instrument is typically equipped with bandpass filter of 400 and 450 nm (70 nm bandwidth) (Corion Co.) for monitoring monomer and eximer fluorescence respectively. An argon ion laser (360 nm 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 dependant 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. A blank prepared.

1.) The sample of cells in culture or cell suspension (200 μL) is incubated with a solution of either perfusion buffer[3] or media containing from 2 - 20 μM fluorescent probe (i.e. 20 - 200 μL Fluorescent Lipid Reagent[1]/ mL of buffer or media). Normally, a 2 μM solution is sufficient for labeling. NOTE: To improve weak or insufficient labeling, 0.08% Pluronic F127 (50 mg provided[4]) (TEFLABS) may be added to these labeling solutions.

2.) Incubate/mix for a 20 minutes at 25ºC, with rotation for cell suspensions, to make sure the reaction system is homogeneous.

3.) The unincorporated pyrenedecanoic acid is removed by two washes of the cells with either perfusion buffer or media.

4.) The cells are resuspended or bathed in media.

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

6.) Read fluorescence at both 400 and 450-470 nm using the appropriate laser emission or excitation filter for excitation at 360 nm. Use reference standard[2] 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

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

8.) Generate a calibration curve by plotting the normalized* fluorescence ratio (Ie/Im) vs. PDA concentration (log-log).

9.) 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.

10.) Additional information on these and alternate protocol conditions is given in the references listed below.

REFERENCES

1.) Yuli, I., et al., Proc. Natl. Acad. Sci. USA **79** (1982) 5906.
2.) Oliver, J., Amer. J. Pathology **93** (1978) 221.
3.) Tomonaga, A., et al., Microbiol. Immunol. **27** (1983) 961.
4.) Haak, R.A. et al., J. Clin Invest. **64** (1979) 138.
5.) Tonooka, T., et al., Pediatric Res. **13** (1979) 148.
6.) Neufeld, N.D., Corbo, L.M., Pediatric Res. **18** (1984) 773.
7.) Masuda, M., J. Immunol. Meth. **96** (1987) 225-231.
8.) Dix, J.A., Verkman, A.S., Biochemistry **29** (1990) 1949.

M0271 KIT CONTENTS			
DESCRIPTION	**QUANTITY**	**PART NO.**	**STORAGE**
REAGENTS			
1 FLUORESCENT LIPID REAGENT [100 μM PYRENEDECANOIC ACID/ 0.1 M PHOSPHATE BUFFER (PH 7.4)]	1 x 2 mL VIAL	0271-001	C, L
2 REFERENCE STANDARD (2mM PYRENE IN DMSO)	1x 2 mL vial	0271-002	C
3 PERFUSION BUFFER	1 x 25 mL VIAL	0271-003	C
4 PLURONIC F127	1 x 50 mg VIAL	0271-004	RT
DOCUMENTATION			
MSDS SHEETS	4	0271-005/6/7/8	N/A
PRODUCT INFORMATION SHEET	1	0271-009	N/A

Notes: F=store at or below -20^{o}C; RT=store at room temperature; C=store cold (4^{o}C); L=light sensitive; FL=flammable; R=read protocol instructions carefully prior to use.

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