INSTRUCTIONS

Chrometra Lipid ExM, Membrane ExM

Polymerizable Fluorescent Lipid derivatives

Product Numbers	Description
LL-1 range	Reagent for staining of lipid membranes in microscopy. **Contents:** Fluorescent lipid derivatives (100 or 300 reactions) **Storage** Upon receipt store product at -20°C.

Introduction

Chrometra Lipid ExM for Membrane staining in polymeric gels

The Chrometra Lipid ExM kit is used to introduce fluorescent labels into fluorescent membranes. By including a polymerizable moiety into the structure, the fluorescent conjugate becomes permanently bound to the surrounding matrix upon polymerization.

Kit Contents

- 1 or 3 vials of fluorescent lipid reagent (100 or 300 reactions, 30 reactions for samples).

Reagent preparation

The fluorescent lipid comes dry and should be suspended as a DMSO or Methanol solution reagent. To this end, add 100 microliter of solvent to the tube and incubate at 37°C while shaking, for five minutes. Prior to staining, 1 µl of this solution is diluted in 50 microliter of suitable buffer (e.g.; PBS, not provided)

Procedure

1. Permeabilize cells with 0.5% Tween at room temperature for 10 minutes.
2. Wash the cells with PBS buffer (3 times, for 5 minutes)
3. Stain the cells for 1h with 50 microliter of fluorescent lipid reagent
4. Wash the cells for 5 minutes with 1xPBS
5. The cells are now ready for further treatment and microscopy

Post Gelation staining

Additional Materials

- 1x PBS
- Matched staining reagent (e.g. ExM post fluorescent stain, a cyclooctyne dye or similar)

Protocol

1. Gelated and digested specimens were prepared in line with the general grafting protocol, with a reactive group incorporated.
2. After digestion, gels were rinsed with 1x PBS once
3. Matched staining reagent (2 ml, 5 µM in PBS) is added. Mix the reagent by gentle pipetting.
4. The sample is incubated at 37°C for 1h, or overnight at room temperature.
5. Wash the gel with PBS buffer (3 times)
6. The gel is now ready for imaging or swelling.

Storage and Safety

The fluorescent lipid can be stored at 4°C for 3 months. For longer storage the conjugate can be stored at -20°C. The conjugate should always be stored in the dark.

Tips and troubleshooting

- To avoid photobleaching of dilute dyes, minimize the exposure of fluorescently labeled specimens to light.
- Inefficient incorporation of the dye stained membranes in the polymeric matrix can contribute to low signal after sample clearing and swelling. When low signal intensity is observed, grafting and cross-linking benefits from gentle polymerization (through the addition of 4-hydroxy TEMPO) and prolonged reaction times.