

INSTRUCTIONS

Chrometra Actin ExM

Polymerizable Fluorescent Phalloidin derivatives

Product Numbers	Description
PL-3-01 to PL-3-05	Reagent for staining of actin in microscopy. Contents: Fluorescent phalloidin derivative (20, 100 or 300 reactions (3 x100 rxn)) Storage Upon receipt store product at -20°C. Product is shipped at room temperature

Introduction
Chrometra Actin ExM for Actin staining in polymeric gels

Fluorescent phalloidin and phalloidin derivatives efficiently label F-actin at nanomolar concentrations. By including a polymerizable moiety into the structure, the fluorescent conjugate becomes permanently bound to the surrounding matrix upon polymerization.

Additional Materials Required

- Methanol
- PBS Buffer
- Triton X-100

Storage and Safety

Once reconstituted in methanol, the stock solutions are stable for at least 6 months when stored frozen at –20°C, desiccated, and protected from light. A loss of activity is exhibited when stored in aqueous solution at 2–6°C for over three weeks.

Preparing the Stock Solution

The vial contents should be dissolved in methanol to yield a final concentration of 200 units/mL, which is equivalent to approximately 6.6 μ M. Here, one unit of phalloidin is defined as the amount of material used to stain one microscope slide of fixed cells, according to the following protocol, and is equivalent to 5 μ L of methanolic stock solution for the fluorescent phalloidins.

<u># of reactions</u>	<u>Methanol Volume needed (μl)</u>
20	100
100	500

Procedure

1. Dilute the phalloidin derivative to form the Stock solution
2. Cells are fixed in 4% PFA in PBS for 10 minutes and washed 3x for 5 minutes with 1xPBS before permeabilization with 0.2% Triton X-100 in PBS at room temperature for 3-15 minutes
3. Mix thoroughly by shaking
4. Dilute 5 μ L methanolic stock solution into 200 μ L PBS for each coverslip to be stained. For a stronger signal, 2-3 units can be added to each coverslip.
5. Incubate for 1h at room temperature.
6. Wash with PBS before imaging and/or polymerization

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 can be prepared in line with general acrylate based hydrogels, and with a reactive group incorporated according to the procedure above.
2. After digestion, gels were rinsed with 1x PBS once
3. Matched staining reagent (2 ml, 5 μ M 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.

Tips and troubleshooting

- Further addition of crosslinker (e.g. Acryloyl X, 10 equivs. in the polymerization step) has been reported to increase S/N in several sample types
- To avoid photobleaching of dilute dyes, minimize the exposure of fluorescently labeled specimens to light.
- In recent versions of the product, methanol has replaced DMSO as solvent of choice. DMSO and DMF can be used, without further changes to the protocols
- Higher stock concentrations can be prepared and used without further changes to the protocol.
- Inefficient incorporation of the dye in the polymeric matrix can contribute to low signal after sample clearing and swelling. When low signal intensity is observed, increase the volume of phalloidin reagent added. In addition, grafting cross-linking benefits from gentle polymerization (through the addition of 4-hydroxy TEMPO) and prolonged reaction times.
- To reduce nonspecific background staining with these conjugates, add 1% bovine serum albumin (BSA) to the staining solution.

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