Microfilament Staining Solution (R250 Method)

M1511591

Storage: 2-8°C, Room Temperature. Store in the dark.
Shipping: Shipped with ice packs.

Introduction:

Cytoskeleton generally refers to the interlaced fiber network in the cytoplasm of eukaryotic cells. According to differences in fiber diameter, components and assembly structure, it can be divided into microtubules, microfilaments and intermediate filaments. Methods for observing the cytoskeleton include electron microscopy, histochemistry, enzyme labeling, immunofluorescence, etc. Microfilaments are fibers composed of actin. Together with certain binding proteins, microfilaments form different subcellular structures in various types of cells (such as muscle filaments, the core of intestinal epithelial microvilli, stress fibers, etc.).

Microfilament Staining Solution (R250 Method) mainly uses Coomassie Brilliant Blue R250 to reveal stress fibers composed of microfilaments. Coomassie Brilliant Blue R250 can stain a variety of proteins and is not specific to microfilaments. Under the conditions of this method, due to the instability of microtubule structure and the fact that some types of fibers are too thin to be recognized under an optical microscope, the fibers visible are mainly stress fibers composed of microfilaments with a diameter of approximately 40 nm. These fibers are particularly prominent in cultured adherent cells in vitro due to the cell's attachment to the culture substrate and maintenance of a flat, spread morphology.

This reagent is for research use only and not intended for clinical diagnostic or other purposes.

Product Components and Storage Conditions:

M1511591	Component	5×50mL	Storage
M1511591A	PBS Buffer(10×)	50mL	RT
M1511591B	TM Buffer	50mL	2-8℃. Store in the dark.
M1511591C	M Buffer(3×)	50mL	2-8℃. Store in the dark.
M1511591D	Microfilament Fluid	50mL	2-8℃. Store in the dark.
M1511591E	R250 Stain	50mL	RT

Protocol (For Reference Only):

1 Microfilaments in Animal Cells:

(1) Sample preparation: Culture cells on a coverslip. When the cell density reaches 60–70%, place the coverslip in a weighing bottle with the cell side up. Dilute PBS Buffer(10×) to 1× with deionized water, wash with 1× PBS Buffer for 1 min, and repeat once.

(2) Extraction: Discard the PBS Buffer, add 2 mL of TM Buffer, cover the weighing bottle, and

incubate at 37 °C for 25–30 min.

(3) Rinsing: Discard the TM Buffer. Dilute 3× M Buffer to 1× with deionized water, wash with 1× M Buffer for 2 min, and repeat twice.

(4) Fixation: Air-dry slightly, add 2 mL of Microfilament Fluid, and fix the cells for 15–20 min.

(5) Washing: Discard the Microfilament Fluid, gently wash with 1× PBS Buffer for 2 min, and repeat once.

(6) Staining: Discard the PBS Buffer. Stand the coverslip on absorbent filter paper to remove excess water from the edges, add 2 mL R250 stain, and stain for 20–25 min.

(7) Rinse off the stain with deionized water, blot dry with filter paper, air-dry, and examine under a microscope or mount with resin.

2 Microfilaments in Plant Cells:

(1) Sample preparation: Dilute PBS Buffer (10×) to 1× with deionized water. Gently tear off approximately 1 cm² of onion bulb epidermis, place it into a weighing bottle prefilled with 1× PBS Buffer, and incubate for 5–10 min until it sinks.

(2) Extraction: Discard the PBS Buffer, add 2 mL of TM buffer, cover the weighing bottle, and incubate at 37 °C for 30 min.

(3) Rinsing: Discard the TM Buffer. Dilute 3× M Buffer to 1× with deionized water, wash with 1× M Buffer for 3–5 min, and repeat twice.

(4) Fixation: Air-dry slightly, add 2 mL of Microfilament Fluid, and fix the cells for 20–25 min.

(5) Washing: Discard the Microfilament Fluid, wash with 1× PBS Buffer for 3–5 min, and repeat twice.

(6) Staining: Discard the PBS Buffer. Stand the coverslip on absorbent filter paper to remove excess water from the edges, add 2 mL R250 stain, and stain for 20–25 min.

(7) Rinse off the stain with deionized water, mount the specimen on a glass slide, cover with a coverslip, and examine under a microscope.

Staining Results:

Under an optical microscope, the outline of animal cells can be observed. Stress fibers appear dark blue, long and straight, often parallel to the long axis of the cell and extending through the entire length of the cell. The outline of onion epidermal cells is clear, and the microfilament bundles appear dark blue. Under high magnification, by adjusting the fine focus, the three-dimensional structure of the cytoskeleton can be seen.

Precautions:

1. Extraction, fixation, and staining should be performed in a covered weighing bottle, with the cell-bearing side of the coverslip always facing upward.
2. Add all reagents slowly along the inner wall of the weighing bottle to avoid direct contact with the coverslip or specimen. Handle cell washing gently to prevent cell detachment.
3. The extraction time should be optimized by the user: excessive time may damage cell structures, while insufficient time may result in high background.

4. Stress fibers are dynamic structures. When cells are fully adherent, the fibers are straight and abundant; otherwise, cells contract and round up, and stress fibers become bent, partially depolymerized, disappeared, or sparse.
5. For your safety and health, please wear a lab coat and disposable gloves during operation.