微丝染色试剂盒(R250 法)

货号： G3560
规格： 5×20 mL/5×50 mL
保存： 2-8℃, 避光保存, 有效期 1 年。

产品组成：

名称	5×20 mL	5×50 mL	保存
试剂(A): PBS Buffer（10×）	20 mL	50 mL	室温
试剂(B): TM Buffer	20 mL	50 mL	室温
试剂(C): M Buffer（3×）	20 mL	50 mL	室温
试剂(D):微丝固定液	20 mL	50 mL	2-8℃，避光
试剂(E): R250 染色液	20 mL	50 mL	室温

产品介绍：

细胞骨架一般是指真核细胞胞质中纵横交错的纤维网，根据纤维直径、组成成分和组装结构的 不同分为微管、微丝和中间纤维。观察细胞骨架的方法有电镜、组织化学、酶标记、免疫荧光等。 微丝是由肌动蛋白构成的纤维，微丝在不同种类的细胞中与某些结合蛋白一起形成不同的亚细胞结 构（如肌肉细丝、肠上皮微绒毛轴心、应力纤维等）。

微丝染色试剂盒(R250 法)主要利用考马斯亮蓝 R250 显示由微丝构成的应力纤维。考马斯亮蓝 R250 可以对多种蛋白染色，并非特异染微丝。在该方法条件下，由于微管结构不稳定，有些类型的纤维 太细，光学显微镜下无法辨认，因此能够看到的纤维主要是由微丝构成的应力纤维，直径约 40nm。 由于细胞对培养基质的附着和维持扁平铺展的形状，该纤维在体外培养的贴壁细胞中尤其发达。

操作步骤：（*仅供参考*）

一、 动物细胞微丝

1. 取材：在盖玻片上细胞培养，生长密度达 60~70% 时细胞面朝上置于称量瓶中。用去离子水稀释 PBS Buffer（10×）至 1×，用 PBS Buffer（1×）清洗 1min，重复 1 次。
2. 抽提：弃 PBS Buffer（1×），加入 2 mL TM Buffer，盖上称量瓶盖子，37℃处理 25~30 min。
3. 漂洗：弃 TM Buffer，用去离子水稀释 M Buffer（3×）至 1×，用 M Buffer（1×）清洗 2 min，重复 2 次。
4. 固定：稍微晾干，加入 2 mL 微丝固定液固定细胞 15~20 min。
5. 冲洗：弃微丝固定液，用 PBS Buffer（1×）轻轻清洗 2 min，重复 1 次。
6. 染色：弃 PBS Buffer（1×），用吸水滤纸吸去称量瓶底和侧壁边缘水分，加入 2 mL R250 染色液，染色 20~25 min。
7. 去离子水冲洗染液，滤纸吸干水分，晾干，镜检或树脂封片。

二、 植物细胞微丝

1. 取材：用去离子水稀释 PBS Buffer（10×）至 1×。轻轻撕取约 1 cm^2 洋葱鳞茎内皮，置于预先加入 PBS Buffer（1×）的称量瓶中，孵育 5~10 min，使其下沉。
2. 抽提：弃 PBS Buffer（1×），加入 2 mL TM Buffer，盖上称量瓶盖子，37℃处理 30 min。
3. 漂洗：弃 TM Buffer，用去离子水稀释 M Buffer（3×）至 1×，用 M Buffer（1×）清洗 3~5 min，重复 2 次。
4. 固定：稍微晾干，加入 2 mL 微丝固定液固定细胞 20~25 min。
5. 冲洗：弃微丝固定液，用 PBS Buffer（1×）清洗 3~5 min，重复 2 次。
6. 染色：弃 PBS Buffer（1×），用吸水滤纸吸去称量瓶底和侧壁边缘水分，加入 2 mL R250 染色液，染色 20~25 min。
7. 去离子水冲洗染液，标本铺在载玻片上，加盖玻片，镜检。

染色结果：

1. 光学显微镜下可见动物细胞轮廓，应力纤维呈深蓝色，形态长而直，常与细胞的长轴平行并贯穿细胞全长。
2. 洋葱表皮细胞轮廓清晰，微丝束呈深蓝色，高倍镜下，转到微调，可见细胞骨架的立体结构。

注意事项：

1. 抽提、固定、染色应在加盖的称量瓶中进行，并且盖玻片的细胞面始终朝上。
2. 沿称量瓶内壁缓慢加入各种试剂，避免直接滴到玻片或样本上；清洗细胞动作应轻柔，避免细胞脱落。
3. 抽提时间应自行摸索，时间过长易破坏细胞结构，时间过短易出现高背景。
4. 应力纤维是一种动态结构，细胞充分贴壁时纤维挺拔、丰富；反之，细胞收缩变圆，应力纤维弯曲甚至部分解聚消失而显得稀少。
5. 本产品仅供科研使用。请勿用于医药、临床诊断或治疗，食品及化妆品等用途。请勿存放于普通住宅区。
6. 为了您的安全和健康，请穿好实验服并佩戴一次性手套和口罩操作。

Microfilament Stain Kit(R-250 Method)

Cat: G3560
Size: 5×20 mL/5×50 mL
Storage:2-8℃, avoid light, valid for 1 year.

Kit components

Reagent	5×20 mL	5×50 mL	Storage
Reagent(A): PBS Buffer(10×)	20 mL	50 mL	RT
Reagent(B): TM Buffer	20 mL	50 mL	RT
Reagent(C): M Buffer(3×)	20 mL	50 mL	RT
Reagent(D): Microfilament Fixative	20 mL	50 mL	2-8℃, avoid light
Reagent(E): R250 Staining Solution	20 mL	50 mL	RT

Introduction

Cytoskeleton generally refers to the crisscross fiber network in the cytoplasm of eukaryotic cells. It is divided into microtubules, microfilaments and intermediate fibers according to the fiber diameter, composition and assembly structure. The methods of observing cytoskeleton are electron microscopy, histochemistry, enzyme labeling, immunofluorescence and so on. Microfilaments are fibers composed of actin. Microfilaments form different subcellular structures with some binding proteins in different kinds of cells (such as muscle filaments, intestinal epithelial microvilli axis, stress fibers, etc.).

Microfilament Stain Kit(R-250 Method)mainly uses Coomassie brilliant blue R250 to display the stress fibers composed of microfilaments. Coomassie brilliant blue R250 can stain a variety of proteins, not specific microfilaments. Under the condition of this method, due to the unstable microtubule structure, some types of fibers are too thin to be identified under the optical microscope, so the fibers that can be seen are mainly stress fibers composed of microfilaments, with a diameter of about 40 nm. Due to the attachment of cells to the culture matrix and maintaining the flat spreading shape, the fiber is particularly developed in adherent cells cultured in vitro.

Protocols*(for reference only)*

For animal cell microfilament

1. Sampling: culture the cells on the cover glass. When the growth density reaches 60-70%, place the cells in the weighing bottle face up. Dilute PBS Buffer (10×) with distilled water to PBS Buffer(1×), wash with PBS Buffer(1×) for 1min and repeat once.
2. Extraction: discard PBS Buffer(1×), add 2 ml TM Buffer, cover the weighing bottle, and treat at 37 ℃ for 25-30 min.
3. Rinsing: discard TM Buffer and dilute M Buffer(3×) with distilled water to M Buffer(1×), wash with M Buffer(1×) for 2 min and repeat twice.
4. Fixation: dry slightly, add 2 ml Microfilament Fixative and fix the cells for 15-20 min.
5. Rinse: discard Microfilament Fixative and gently wash with PBS Buffer(1×) for 2 min and repeat once.
6. Dyeing: discard the PBS Buffer(1×), absorb the excess water on the bottom and side wall by absorbent filter paper, then add 2 ml R250 Staining Solution and stain for 20-25 min.
7. Wash the solution with distilled water, dry it with filter paper, and conduct microscopic inspection or resinene sealing.

For plant cell microfilament

1. Sampling: dilute PBS Buffer (10×) with deionized water to PBS Buffer(1×). Gently tear about 1 cm^2 onion bulb endothelium and place it in PBS Buffer (1×). Incubate in a weighing bottle for 5-10min to sink.
2. Extraction: discard PBS Buffer (1×), add 2 ml TM Buffer, cover the weighing bottle and treat at 37 ℃ for 30 min.
3. Rinsing: discard TM Buffer and dilute M Buffer(3×) with distilled water to M Buffer(1×), wash with M Buffer(1×) for 3-5 min and repeat twice.
4. Fixation: dry slightly, add 2 ml Microfilament Fixative and fix the cells for 20-25 min.
5. Rinse: discard Microfilament Fixative and gently wash with PBS Buffer(1×) for 3-5 min and repeat twice.
6. Dyeing: discard PBS Buffer(1×), absorb the excess water on the bottom and side wall by absorbent filter paper, then add 2 ml R250 Staining Solution and stain for 20-25 min.
7. Rinse the dye solution with distilled water, pick the specimen on the slide, cover the slide and conduct

microscopic examination.

Result

1. The outline of animal cells can be seen under the optical microscope. The stress fibers are dark blue, long and straight, often parallel to the long axis of the cells and throughout the whole length of the cells.
2. The outline of onion epidermal cells is clear, and the microfilament bundles are dark blue. Under high-power microscope, turn to fine-tuning, and the three-dimensional structure of cytoskeleton can be seen.

Note

1. Extraction, fixation and staining shall be carried out in a capped weighing bottle, and the cell surface of the cover glass shall always face upward.
2. Slowly add various reagents along the inner wall of the weighing bottle to avoid dropping directly on the glass slide or sample; Clean cells gently to avoid cell shedding.
3. The extraction time should be explored by ourselves. Too long time is easy to destroy the cell structure, and too short time is easy to produce high background.
4. Stress fiber is a dynamic structure. When the cells fully adhere to the wall, the fibers are tall and rich; On the contrary, cells shrink and become round, stress fibers bend and even partially depolymerize and disappear.
5. This product is only for scientific research. Do not use for medicine, clinical diagnosis or treatment, food and cosmetics. Do not store in ordinary residential areas.
6. For your safety and health, please wear lab clothes and disposable gloves and masks.