改良番红 O-固绿软骨染色试剂盒

货号： G1371
规格： 5×50mL/5×100mL
保存： 室温，避光保存，有效期 1 年。

产品组成：

名称		5×50mL	5×100mL	保存
试剂(A)：Weigert 铁苏木素染色液	A1:Weigert A 液	25mL	50mL	室温，避光
	A2:Weigert B 液	25mL	50mL	室温，避光
临用时，取 A1、A2 等量混合为 Weigert 染液，24h 后失去染色力，不宜预先配制。				
试剂(B): 酸性分化液		50mL	100mL	室温
试剂(C): 固绿染色液		50mL	100mL	室温，避光
试剂(D): 番红染色液		50mL	100mL	室温，避光
试剂(E): 弱酸溶液		50mL	100mL	室温

产品介绍：

软骨组织由软骨细胞、软骨基质和纤维组成，软骨组织及其周围的软骨膜构成软骨。软骨根据基质内所含纤维素成分不同分为透明软骨、弹性软骨、纤维软骨。软骨染色方法有很多种，例如甲苯胺蓝法、阿利新蓝法、番红 O 法等。

改良番红 O-固绿软骨染色法的染色原理在于嗜碱性的软骨与碱性染料番红 O 结合呈现红色，嗜酸性的骨和酸性染料固绿结合而成绿色或蓝色，与呈现红色的软骨对比鲜明，从而将软骨组织和骨组织区分开。番红 O 着色与阴离子的浓度近似成正比关系，间接反映了基质中蛋白多糖的含量和分布。当软骨受到损伤时，软骨中的糖蛋白会释放出来，使基质成分分布不均匀，从而导致番红 O 淡染或不着色。通过图像分析软件可对番红 O 染色的软骨基质进行定量分析。固绿与胶原纤维结合，不易褪色。番红 O-固绿染色的分化很关键，分化过度易导致切片不着色，分化不足易导致切片着色过深。

自备材料：

10%福尔马林固定液，脱钙液，蒸馏水，系列乙醇。

操作步骤：(*仅供参考*)

1. 标本的处理：10%福尔马林固定、脱钙、石蜡切片。
2. 常规脱蜡至水。
3. 入新鲜配制的 Weigert 染液染色 3-5min，水洗。
4. 酸性分化液分化 15s。蒸馏水洗 10min。
5. 在固绿染色液内浸染 5min。
6. 快速用弱酸溶液洗涤切片 10-15s，以便去除残留的固绿，晾干。
7. 入番红染色液内浸染 5min。
8. 按 95%乙醇 2-3s、无水乙醇 2-3s，无水乙醇 1min 脱水。
9. 二甲苯透明，光学树脂封固。

染色结果：

软骨基质	深红色
软骨细胞核	蓝色
细胞浆、肌肉、胶原纤维及骨组织	灰绿色
软骨细胞浆	红色
细胞核	灰黑色

注意事项：

1. 需要显示细胞核时，尽量采用铁苏木素染色，其着色力强色调浓，一般的苏木素着色力不强。

2. Weigert 苏木素染液不可预先配制后放置，配制好后一般 24h 失去染色能力。
3. 切片在番红染色液中染色不宜过长，否则易导致背景的深红色不易分化掉。
4. 切片分化时间应恰当，以背景呈绿色为宜。
5. 番红染色液染色后，不宜在低浓度乙醇脱水，否则易褪色。
6. 95%乙醇脱水时间不宜过长。
7. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Modified Saffron-O and Fast Green Stain Kit

Cat: G1371
Size: 5×50mL/5×100mL
Storage: RT, avoid light, valid for 1 year.

Introduction

Cartilage tissue is composed of chondrocytes, cartilage matrix and fibers, and cartilage tissue and its surrounding cartilage membrane constitute cartilage. Cartilage can be divided into hyaline cartilage, elastic cartilage and fibrocartilage according to the cellulose content in the matrix. There are many methods of cartilage staining, such as Toluidine Blue Method, Alixin Blue Method, Safranine O Method and so on.

The dyeing principle of the Modified Saffron-O and Fast Green Stain Kit is that the combination of the basophil cartilage and the basic dye Saffron-O is red, and the eosinophilic bone and the acid dye Fast Green is green or blue, which is in sharp contrast with the red cartilage, so as to distinguish the cartilage from bone tissue. Saffron-O staining is approximately proportional to the concentration of anions, which indirectly reflects the content and distribution of proteoglycans in the matrix. When cartilage is damaged, glycoprotein in cartilage will be released to make uneven distribution of matrix components, resulting in light or non staining of Saffron-O. The cartilage matrix stained with Saffron-O can be quantitatively analyzed by image analysis software. Fast Green combines with collagen fibers, which is not easy to fade. The differentiation of Saffron-O-Fast Green staining is very important. Excessive differentiation is easy to lead to no staining of sections, and insufficient differentiation is easy to lead to too deep staining of sections.

Kit Components

Reagent		5×50mL	5×100mL	Storage
Reagent(A):Weigert's Iron Hematoxylin Solution	A1:Weigert Solution A	25mL	50mL	RT, avoid light
	A2:Weigert Solution B	25mL	50mL	RT, avoid light
Mix equal parts of A1 and A2 to form Weigert Hematoxylin Working Solution before use. This working solution is stable for about 24 hours and not suitable to prepare in advance.				
Reagent(B): Acid Differentiation Solution		50mL	100mL	RT
Reagent(C): Fast Green Staining Solution		50mL	100mL	RT, avoid light
Reagent(D): Saffron-O Staining Solution		50mL	100mL	RT, avoid light
Reagent(E): Weak Acid Solution		50mL	100mL	RT

Self Provided Materials

10% formalin fixative, decalcifing solution, distilled water, series of ethanol.

Protocol(*for reference only*)

1. Speciman Treatment: fix in 10% formalin fixative, decalcification, make paraffin section.
2. Dewax to distilled water.
3. Stain with Weigert Hematoxylin Working Solution for 3-5mins and then wash with water.
4. Differentiate with Acid Differentiation Solution for 15s. Rinse in distilled water for 10mins.
5. Stain in Fast Green Staining Solution for 5mins.
6. Rinse in Weak Acid Solution for 10-15s to remove the remaining Fast Green Staining Solution. Air dry.
7. Stain in Saffron-O Staining Solution for 5mins.
8. Dehydrate in 95% ethanol for 2-3s, absolute ethanol(I) for 2-3s and absolute ethanol(II) for 1 min.
9. Transparent by xylene and seal with optical resin.

Result

Cartilage Matrix	Deep Blue
Cartilage Nucleus	Blue
Cytoplasm, Muscle, Collagen Fiber and Bone Tissue	Grey Green
Cartilage Cytoplasma	Red
Nucleus	Grey Black

Note

1. When it is necessary to show the nucleus, it is best to use Iron Hematoxylin Staining, which has strong

coloring power and thick color. General hematoxylin staining power is not strong.

2. Weigert hematoxylin Working Solution can not be placed after preparation in advance. This working solution is only stable for about 24 h.
3. The staining time in Saffron-O Staining Solution should not be too long, otherwise it is easy to cause the dark red of the background and not easy to differentiate.
4. The time of differentiation should be appropriate and the background should be green.
5. After Saffron-O Staining Solution dyeing, it is not suitable to dehydrate in low concentration ethanol, otherwise it is easy to fade.
6. The dehydration time of 95% ethanol should not be too long.
7. For your safety and health, please wear experimental clothes and disposable gloves.