Modified Safranin O-Fast Green Cartilage Staining Kit

Catalog No.: RA20095

Basic Information

Product name	Modified Safranin O-Fast Green Cartilage Staining Kit
Sizes	50 mL, 100 mL
Storage	RT
Shipping	RT
Validity	12 months

Product Introduction

Cartilage tissue is composed of chondrocytes and cartilage matrix. Cartilage tissue and its surrounding perichondrium constitute cartilage. Based on the fiber components in the matrix, cartilage is classified into hyaline cartilage, elastic cartilage, and fibrocartilage. There are various cartilage staining methods, such as toluidine blue, Alcian blue, and Safranin O methods.

The staining principle of EnkiLife Modified Safranin O-Fast Green Cartilage Staining Method is that basophilic cartilage binds to the basic dye Safranin O, appearing red, while acidic bone tissue binds to the acidic dye Fast Green, appearing green or blue. The contrast between red cartilage and green/blue bone tissue allows clear differentiation. Safranin O is a cationic dye that binds to polyanions. It stains cartilage by binding to anionic groups (e.g., chondroitin sulfate or keratan sulfate) in proteoglycans. The intensity of Safranin O staining is approximately proportional to the concentration of anions, indirectly reflecting the content and distribution of proteoglycans in the matrix. When cartilage is damaged, glycoproteins are released, leading to uneven matrix composition, resulting in weak or no staining by Safranin O. Quantitative analysis of Safranin O-stained cartilage matrix can be performed using image analysis software. Fast Green binds to collagen fibers and is resistant to fading. Differentiation is critical in Safranin O-Fast Green staining: over-differentiation may lead to no staining, while under-differentiation may result in overly intense staining.

Product Components

Components		5x 50mL	5x 100mL
Reagent (A):	A1: Weigert Solution A	25 mL	50 mL
	A2: Weigert Solution B	25 mL	50 mL

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Mix A1 and A2 in equal parts before use. Do not prepare in advance. Weigert stain loses activity after 24 h.		
Reagent (B): Acid Ethanol Differentiation Solution	50 mL	100 mL
Reagent (C): Fast Green Staining Solution	50 mL	100 mL
Reagent (D): Acetic Acid Solution	50 mL	100 mL
Reagent (E): Safranin O Staining Solution	50 mL	100 mL

Materials Required (Not Supplied)

1. 10% formalin fixative, decalcifying solution, distilled water, graded ethanol series.
2. Xylene or eco-friendly deparaffinizing and clearing solution, neutral balsam or eco-friendly mounting medium.

Experimental procedure

1. Sample preparation: Fix with 10% formalin, decalcify, embed in paraffin, section, and deparaffinize with xylene or substitute to water.
2. Apply freshly prepared Weigert stain for 3–5 min, then rinse with water.
3. Differentiate with acid ethanol differentiation solution for 15 s, then rinse with distilled water for 5–10 min.
4. Apply Fast Green staining solution for 1–5 min.
5. Quickly rinse with acetic acid solution for 10 – 15 s to remove residual Fast Green. Air-dry (or differentiate briefly with acid ethanol for 10–15 s, then rinse with tap water).
6. Apply Safranin O staining solution for 2–5 min.
7. Quickly dehydrate with absolute ethanol 4 times, 3–5 s each. Check under microscope after the 4th dehydration: cartilage should appear red, background should be colorless.
8. Clear with xylene or substitute, mount with neutral balsam, examine under microscope, and perform image acquisition and analysis.

Staining Results

Component	Color
Cartilage matrix	Red, orange-red, or deep red

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Chondrocyte nuclei	Blue
Chondrocyte cytoplasm	Red
Cytoplasm, muscle, collagen fibers, and osteoid tissue	Gray-green
Nuclei	Blue-purple to deep blue

Notes

1. To visualize nuclei, iron hematoxylin is recommended due to its strong staining intensity and deep tone. Ordinary hematoxylin may not stain strongly enough.
2. Weigert stain should not be prepared in advance; it loses staining activity after 24 h.
3. If large volumes of acetic acid solution are needed, prepare 0.05–0.1% acetic acid aqueous solution as substitute.
4. Do not overstain with Safranin O, as it may mix with green and produce a purple-blue color.
5. Do not arbitrarily extend staining time after Safranin O application.
6. For your safety and health, wear a lab coat and disposable gloves during operation.

This product is for research use only!